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# EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Seventieth report of the
Joint FAO/WHO Expert Committee on
Food Additives

Food and Agriculture Organization of the United Nations

World Health Organization

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 the Joint FAO/WHO Expert Committee on Food Additives (JECFA), including a hypothesis-driven decision tree approach for the safety evaluation of residues of veterinary drugs; comments on the Committee for Veterinary Products for Medicinal Use reflection paper on the new approach developed by JECFA for exposure and maximum residue limit (MRL) assessment of residues; residues of veterinary drugs in honey and possible approaches to derive MRLs for thiscommodity; comments on a paper entitled "Risk-assessment policies: Differences among jurisdictions"; and the use of no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL) in JECFA assessments.

Summaries follow of the Committee's evaluations of toxicological and residue data on a variety of veterinary drugs: three antimicrobial agents (avilamycin, tilmicosin, tylosin), one anthelminthic (triclabendazole), one production aid (melengestrol acetate), two antimicrobial agents and production aids (monensin and narasin), a glucocorticosteroid (dexamethasone) and an antimicrobial agent and contaminant (malachite green). Annexed to the report is a summary of the Committee's recommendations on these drugs, including acceptable daily intakes (ADIs) and proposed MRLs.

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# Seventieth meeting of the Joint FAO/WHO Expert Committee on Food Additives

Geneva, 21–29 October 2008

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

*Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 61, in press.

Specifications are issued separately by FAO under the title:

*Compendium of food additive specifications.* FAO JECFA Monographs 6, 2009, in press.

**INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY**

The preparatory work for toxicological evaluations of food additives and contaminants by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) is actively supported by certain of the Member States that contribute to the work of the International Programme on Chemical Safety (IPCS).

The IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organization and the World Health Organization. One of the main objectives of the IPCS is to carry out and disseminate evaluations of the effects of chemicals on human health and the quality of the environment.

### Use of JECFA reports and evaluations by registration authorities

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

# 1. Introduction

A meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was held at the World Health Organization (WHO) Headquarters, Geneva, from 21 to 29 October 2008. The meeting was opened by Dr David Heymann, Assistant Director-General, Health Security and Environment, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and WHO. Dr Heymann informed the Committee that the Director-General of WHO, Dr Margaret Chan, has presented an ambitious plan for the Organization, which is summarized in a six-point agenda. Two of these points aim at strengthening health systems and at using evidence to define strategies and measure results. It is in this context that the work of JECFA contributes to the programme of the Organization.

Dr Heymann explained that reorganizations within WHO have taken place to achieve the goals laid out in the six-point agenda. One of these was the formation of the new cluster on Health Security and Environment in October 2007. The new cluster has two elements: access to drugs and vaccines; and public health security with a focus on disease prevention, and in this context food safety plays an important role. In a strategic move to strengthen risk assessment related to food safety within the cluster, the JECFA and Joint FAO/WHO Meeting on Pesticide Residues (JMPR) programmes have been moved to the food safety department.

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

[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-nine previous meetings of JECFA (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 seventieth JECFA meeting had completed declaration of interest forms and that no conflict of interest had been identified.

# 2. General considerations

## 2.1 A hypothesis-driven decision tree approach for the safety evaluation of residues of veterinary drugs

In response to recommendations of the sixty-sixth JECFA (Annex 1, reference *181*), a small working group developed a concept paper entitled "A Hypothesis-Driven Decision Tree Approach for the Safety Evaluation of Residues of Veterinary Drugs" for discussion at the current meeting. The concept paper offers a hypothesis-driven decision tree approach to the risk assessment of residues of veterinary drugs that builds upon the foundation in risk analysis to provide greater flexibility in the advice that JECFA can provide on issues relating to the potential human health effects of residues of veterinary drugs. In particular, it offers possible strategies for assessing compounds for which there are few, if any, sponsors, compounds for which the database is old and/or incomplete, and compounds that are not authorized, where any residues would arise through environmental contamination or illegal use.

The approach recognizes the importance of a preliminary risk assessment to identify the available data and the data gaps and to help refine the risk questions that drive the analysis. The derivation of the risk questions, a step often known as problem formulation, requires close interaction between JECFA and the risk manager seeking advice (e.g. Codex Committee on Residues of Veterinary Drugs in Foods [CCRVDF]). The approach further identifies additional tools and options that may be of value in the evaluation of veterinary drug residues and in the communication of the conclusions on risk to those responsible for risk management. The approach is intended to provide increased consistency and transparency to the evaluation of residues of veterinary drugs while offering greater flexibility and adaptability to changes in technology, science and safety concerns.

An important consideration is that while a decision tree approach based on risk analysis may be anticipated to offer improved flexibility in the evaluation of certain categories of veterinary drugs, the need for sufficient data of adequate quality cannot be overemphasized. However, the decision tree approach

may help to identify critical data gaps early in the evaluation and potentially lead to the generation of suitable data that will permit a successful evaluation of the veterinary drug.

The concepts embodied in the paper were discussed by the Committee. The Committee endorsed the proposal in principle and generally agreed with the concepts as presented. The Committee discussed and made recommendations for the draft, while it emphasized the following general and specific comments:

### *General comments*

— It was agreed that at this early stage of development, the concepts presented are more important than detailed considerations of roles and implementation. It was recognized that there is little guidance currently presented to direct when and how decisions are made among the decision tree options.

— Inherent in the conceptual approach is an early interactive dialogue between risk managers and risk assessors that may not be fully reflected in the current organizational structures.

— The Committee further noted the need to coordinate and, wherever appropriate, reference similar and related efforts, to ensure a comprehensive and non-duplicative approach. These efforts include the FAO/WHO joint Project to Update and Consolidate Principles and Methods for the Risk Assessment of Chemicals in Food, decisions of this and other joint expert committees and the FAO guidance document on risk analysis (*2*).

— Evaluations by the Committee are based on Good Practice in the Use of Veterinary Drugs as defined in the 17th Codex Procedural Manual (*3*). Assessments on the potential risk to human health of exposure as a consequence of other scenarios, such as specified misuse scenarios, would be done only on a specific case-by-case basis. The importance of problem formulation under such circumstances was emphasized.

— Some of the concepts raised were applicable to more areas of risk assessment of chemicals than the assessment of residues of veterinary drugs, and those issues that were cross-cutting should be identified and developed with the respective experts from other bodies.

### *Specific comments*

— The Committee emphasized the importance of clearly presenting the intended purpose of the decision tree approach and identifying the target audiences early in the document.

— The Committee recognized that there are many areas identified in the concept paper that will require additional discussion and development. Aspects related to considerations of acute and chronic exposure were particularly identified as needing development and integration into the decision tree approach.

— There are a number of advanced approaches to risk assessment, such as toxicodynamic modelling, that have not been addressed in the present document. These should be mentioned, even though they may not be readily applicable to the majority of veterinary drugs.

The Committee recommended, in the interest of transparency and communication, that the JECFA Secretariat share the concept paper with relevant FAO/WHO bodies, including joint expert committees and Codex committees, while clearly communicating that this paper is a first step in a long-term project. The Committee further recommended that the JECFA Secretariat convene one or more working groups as appropriate to expand and continue the development of a general decision tree approach for the evaluation of veterinary drugs. Progress in the development of this decision tree approach should be discussed at future JECFA meetings.

The Committee identified the following areas for follow-up work:

— Implementation of proposed steps, such as preliminary risk assessment.

— Approaches for chronic and acute exposure assessments.

— Considerations related to the use of the acute reference dose (ARfD) and associated issues related to short-term exposure.

— Applicability of the threshold of toxicological concern approach to residues of veterinary drugs.

— Exploring ways to leverage resources and increase efficiency with approaches such as work sharing between regulatory bodies and JECFA.

— Specific guidance on the application of the decision tree approach, cross-referenced to the diagrams provided.

— Guidance on the use of default and other data-derived safety factors.

— Applicability of other advanced risk assessment procedures and tools not addressed in the present concept paper.

The draft concept paper will be posted on the JECFA web site.

## 2.2 Comments on the CVMP "Reflection Paper on the New Approach Developed by JECFA for Exposure and MRL Assessment of Residues of VMP"

The Committee considered the Committee for Medicinal Products for Veterinary Use (CVMP) document entitled "Reflection Paper on the New Approach Developed by JECFA for Exposure and MRL Assessment of Residues of VMP [Veterinary Medicinal Products]" (*4*). This document, dated 22 September 2008 and submitted to the JECFA Secretariat, provides comments on the use of the estimated daily intake (EDI) as recommended by the sixty-sixth JECFA (Annex 1, reference *181*).

The Committee acknowledged the comments provided in the reflection paper and noted the history of the development of the EDI and discussion of how it fits into JECFA's evaluation of residues of veterinary drugs, as reported by the sixty-sixth JECFA. The sixty-sixth JECFA identified the EDI as one of a number of issues that are being addressed as part of the FAO/WHO joint Project to Update and Consolidate Principles and Methods for the Risk Assessment of Chemicals in Food. The current Committee confirmed the utility of the EDI as a tool to ensure that intakes of residues resulting from use of veterinary drugs in accordance with Good Practices in the Use of Veterinary Drugs and the recommended MRLs do not exceed the ADI. The Committee acknowledged that the use of the EDI is currently applicable only to the evaluation of chronic toxicity of, and chronic exposure to, residues as reflected by the ADI. The Committee reconfirmed that it requires an adequate data set to estimate the EDI. When the data are not adequate to estimate the EDI, other conservative approaches to ensure that the ADI is not exceeded are applied. Future work will address considerations to identify the appropriate measures of hazard, consumption and exposure for issues of acute toxicity and acute exposure, as might be appropriate for an ARfD. As noted by the sixty-sixth JECFA, the EDI should not be applied when there is concern for acute toxicity or acute exposure. For this purpose, appropriate tools and approaches will need to be developed.

## 2.3 Residues of veterinary drugs in honey and possible approaches to derive MRLs for this commodity

The current Committee considered the guidance regarding honey from the sixty-sixth meeting of the Committee (Annex 1, reference *181*). The sixty-sixth 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 a case, to develop a draft recommendation for consideration at the next meeting. The fifty-second meeting of the Committee (Annex 1, reference *140*) first

considered the subject and requested that the 12th Session of the CCRVDF comment on the matter; however, no formal comments were provided.

The Committee recognized that there are substantial production and trade in honey; however, there are very limited numbers of residue (MRL) standards for residues in honey. Honey production figures for 2005 indicate that approximately 1400 000 tonnes were produced worldwide. Honey production is subject to numerous environmental factors, such as cropping, weather conditions and the impact of pests and pathogens.

### *Points to consider*

The Committee noted that there were a number of factors to consider in developing a process to address the need for recommending MRLs regarding the use of veterinary drugs and pesticides for bee health. They include, but may not be limited to, the following points:

— The recommendations must be within the Committee's terms of reference, with adequate flexibility to meet differing conditions and availability of information.

— There is a need to accommodate a robust yet conservative approach to facilitate MRL recommendations and encourage sponsorship for studies that the Committee considers necessary for recommending MRLs.

— The drug is available as a commercial product, and the commercial product containing the active ingredient is currently registered by a national or regional authority.

— Honey production and honey bees in most countries are considered as a minor use and/or a minor species food product, and the availability of active sponsors to provide studies suitable for recommendations on MRLs is likely to be limited.

— Honey is widely used as a sweetener and glazing agent in confectionery products, breakfast cereals and baked goods, in addition to direct consumption of liquid and set honey, and these uses must be accounted for in intake estimates.

— Several substances used to manage bee health are unlikely to raise public health concerns, because intakes of residues resulting from effective use are far below internationally established ADIs.

— Some proprietary products are not registered for use in bee colonies, and therefore approved dosages and conditions of use do not exist.

— For a number of substances registered for use by national authorities, no contemporary toxicological evaluation may have been performed, or the

review did not result in the establishment of a health-based guidance value, such as an ADI.

— The main groups of substances that typically leave residues in edible bee products are antibiotics (residues in honey and royal jelly) and persistent lipophilic acaricides (residues in wax and propolis).

— Royal jelly should be the subject of a separate and later consideration.

— Residues in both honey and wax need to be considered in exposure estimates. The ratio of honey to wax is typically 9:1.

### *Substances with an existing ADI and/or MRL in a food-producing animal or food commodity*

The main groups of substances that typically leave residues in edible bee products are antibiotics and persistent lipophilic acaricides. Of the products known to be used for treatment of bee diseases, most, but not all, have a national registration and a JECFA or JMPR evaluation with an ADI and/or MRL (or the equivalent in national legislation) for either a food-producing animal or other food commodity, and usually the active ingredients are substances with a long history of use.

### *Substances generally regarded as safe*

Several substances are unlikely to raise public health concerns, because any use in food-producing animals, especially the use in bees, is generally regarded as safe. Examples of such substances include formic acid, lactic acid, oxalic acid, thymol and menthol. In the case of a substance that has clear documentation to support the designation as "generally regarded as safe" by national regulatory authorities and not requiring a MRL, a similar designation can be made. It would require a proviso that equivalence can be demonstrated in honey and that the ADI is sufficient so that no MRL would be required and the ADI is not exceeded. In the case of a new substance not previously considered for registration by national authorities, substances would have to be evaluated as new animal drugs or pesticides and subject to a full food safety risk assessment.

### *Use of non-approved veterinary drugs or pesticides*

In the situation where a substance is not approved for use in food-producing animals (e.g. chloramphenicol or nitrofurans), no exception for honey would be applied.

### *Products used in apiculture*

Table 1 contains a list of products used in apiculture.

Table 1
**List of products used in apiculture**

| Substance | Proprietary product | ADI (mg/kg bw per day) | |
|---|---|---|---|
| | | JECFA | JMPR |
| Acrinathrine | Yes | | |
| Amitraz | Yes | | 0–0.01 |
| Bromopropylate | Yes | | 0–0.03 |
| Chlorobenzilate | No | | 0–0.02 |
| Chlortetracycline | No | 0–0.003 | |
| Coumaphos | Yes | | [a] |
| Cymiazole hydrochloride | Yes | | |
| Enilconazol (imazalil) | No | | 0–0.03 |
| Erythromycin | No | 0–0.0007 | |
| Fenproximate | Yes | | |
| Fipronil | No | | 0–0.0002 |
| Flumethrin | Yes | | 0–0.004 |
| Formic acid[b] | Yes | 0–3 | |
| Fumagillin | Yes | | |
| Lactic acid[b] | No | Not limited | |
| Lincomycin hydrochloride | ? | 0–0.03 | |
| Malathion | No | | 0–0.3 |
| Menthol[b] | Yes | 0–4 | |
| Methyl bromide | No | | |
| Monensin | No | 0–0.01 | |
| Oxalic acid[b] | Yes | | |
| Oxytetracycline | Yes | 0–0.003 | |
| Paradichlorobenzene | No | | |
| Permethrin | Yes | | 0–0.05 |
| Propargite | ? | | 0–0.01 |
| Rifampicin | No | | |
| Spinosad | No | | 0–0.02 |
| Streptomycin/dihydrostreptomycin | No | 0–0.05 | |
| Sulfathiazole | No | No ADI allocated | |
| Tau-fluvalinate | Yes | | |
| Thymol[b] | Yes | Acceptable | |
| Tylosin tartrate | Yes | 0–0.03 | |

bw, body weight
[a] Temporary ADI withdrawn in 1980; no ADI allocated in 1990.
[b] Substances considered by many national authorities as generally regarded as safe.

### *Suggested tools for data generation*

Where an established ADI exists for use in another species as either a veterinary medicine or pesticide, this would generally require a smaller set of additional data for honey, as an ADI exists.

*1. Design criteria for residue data studies*

Study design for residue determinations must take into account how the residue behaves in honey, as well as the following:

— number of apiaries involved, representing a variety of honey types;

— number of hives per apiary sampled;

— number of frames per hive sampled;

— number of samples of wax and honey to be taken from a frame;

— number and spacing of time points to describe the kinetics of formation and depletion of honey in the edible products;

— estimates of amounts of surplus honey present at the beginning of, during and after the treatment until the end of the trial;

— scheme for the analysis of individual and bulk samples;

— climatic information for the duration of the trial, including season of the year (e.g. rainfall);

— crops on which bees forage;

— temperature profile within the hive;

— data on honey flow periodicity;

— data on any supplemental feed given to bees;

— data on bee health and bee/parasite mortality during the study;

— a protocol for the analysis of individual and bulk samples;

— studies on storage stability of residues in honey.

The above factors recognize the unique nature of honey, as all the drugs and metabolites collect in the honey, and the only mechanisms for reduction are dilution as more honey is produced, removal from the hive, and photochemical or thermal degradation of the residues in the honey or through such factors as pH and environmental conditions. Bees commonly move honey around the hive as required, and this can lead to significant variations in residue concentration, even across the same frame in all three hive dimensions.

The quality of the data should allow a statistical evaluation to determine the confidence intervals necessary to recommend the setting of MRLs. The data should show with 95% statistical confidence that 95% of all honey samples from treated bees would be below the MRL and that the estimated intake of residues (considering all other sources of intake) remains below the ADI. As the design of the study depends on many factors, it has to be developed on a product-by-product basis, depending on the use pattern.

*2. Marker residue*

The marker residue concept may not be normally or easily applied to honey scenarios. However, it is important to sufficiently identify and, where feasible, quantify metabolites and degradation products in honey.

### *Dietary intake considerations*

The Committee reviewed the adequacy of its currently used consumption figure of 20 g of honey per day for the estimation of chronic and acute intakes. When the original food basket of the Committee was established, care was taken to ensure that the consumption figures protected the preferential eater of foods of animal origin. Based on data that were available, the 97.5th percentile of daily consumption by the consumers of a commodity from a country with a known high consumption of the commodity was chosen. It was also considered necessary to derive figures that would cover the intake resulting from consumption of processed products containing the raw commodity. For honey, these aspects were not sufficiently addressed at previous meetings of the Committee.

The Committee used a study conducted in 1986–1989 in Germany as a basis for the study of methodological aspects of deducing a figure for daily intake of honey, because in this study consumption data were available for more than 9000 consumers of honey. In addition, more recent data and studies from the United Kingdom, the Netherlands and Germany were reviewed. In the above-mentioned German study, more than 9000 " eaters" of honey had consumed approximately 26 000 portions of honey during a 7-day observation period. The 99.95th percentile of all portions consumed on 1 day was approximately 144 g. This percentile may be considered as an estimate of acute intake, although further guidance on this is needed.

In discussing criteria for the establishment of an estimate of chronic intake, it was concluded that such a figure should be derived from the consumption data for the "chronic" eaters only.

In the German study, the 97.5th percentile of consumption by the subgroup who consumed ≥7 portions in a week was approximately 55 g/day per consumer. The data from the United Kingdom were also based on a 7-day survey

and had been published by the Food Standards Agency. These data were available only in an aggregated format, and the number of participants was lower. However, the highest estimated percentiles were not too different from those from the German study. Data from a 2-day survey in the Netherlands had been evaluated and used as a basis for modelling. The results obtained were significantly lower than the estimates that were based on the United Kingdom and German data.

As the data from the United Kingdom clearly indicated that infants and young children have the highest consumption on a body weight basis, this finding was further investigated. The Committee concluded that this was due mainly to the lower body weight of this group and not to higher consumption. In this context, a recent German survey found that the 97.5th percentile of honey consumption by children 2–5 years of age was 22.1 g/day.

The Committee noted that a consumption figure of 50 g/day per person would most likely protect all groups of consumers; however, further data are necessary to determine whether this figure also sufficiently covers the consumption of products containing honey.

Honey comb, with its original honey content, is consumed by a subgroup of consumers. Many lipophilic substances used as acaricides accumulate in wax. Therefore, the labels of certain registered products warn that wax from bees treated with the products should not be consumed. The Committee concluded that in cases where honey comb can be safely consumed, it would use a honey to wax ratio of 9:1 in the estimation of intakes.

### *Recommendations*

In considering the matters of interest noted in this report and the complex and unique nature of honey and honey bees, JECFA may not be able to adopt any specific approaches without further guidance from CCRVDF. The Committee therefore makes the following recommendations to CCRVDF:

1. That CCRVDF, with the aid of member countries, compile a comprehensive list of all veterinary drugs registered for honey production and bee health and develop a priority list of veterinary drugs for use in honey bees to be considered for risk assessment by JECFA.
2. That CCRVDF and member countries be encouraged to provide data on honey consumption, considering both direct and indirect honey intake, for purposes of improved intake assessments as part of the risk assessment for recommending MRLs.
3. That CCRVDF consider extension of good veterinary practice guidelines to include honey production.

4. That the CCRVDF ad hoc Working Group on Methods of Analysis and Sampling consider analytical methods for residues in honey.
5. That CCRVDF provide guidance on the appropriate percentile for an estimation of acute intake.

The Committee further makes the following recommendation to the JECFA Secretariat:

1. That the JMPR Secretariat be advised of the Committee's report regarding residues in honey and considerations of residues from use of pesticides in honey production and bee health.

## 2.4 Paper by Millstone et al. (2008)—Risk assessment policies: Differences across jurisdictions

In the paper by Millstone et al. (*5*), the Committee found of interest the comparison of risk assessment policies in different jurisdictions, including the joint FAO/WHO bodies such as JECFA. The Committee fully agrees on the need for greater harmonization and transparency in the conduct of risk assessment, which are among the purposes of risk assessment policies. The Committee noted that both risk assessment policies and practice of risk assessment evolve over time and that the methods and principles underpinning the risk assessment work of JECFA and its sister bodies are currently being consolidated and updated, which should ensure greater harmonization. Moreover, expert meetings of WHO and FAO, such as JECFA, are conducted according to well established rules in the respective constitutions of WHO and FAO. In addition, JECFA has embarked upon an activity to increase the flexibility with which it can respond to the needs of the Codex committees and Member States, with respect to concerns about residues arising from veterinary drugs. The Committee was somewhat surprised at the apparent misunderstanding of some of its practices and procedures as reported in the paper by Millstone et al. It is important that readers interested in the workings of JECFA and its sister bodies seek up-to-date guidance from appropriate sources, such as the Secretariat or the WHO and FAO web sites.

There are a number of disparities between the text of Millstone et al. and current practices of JECFA. These include the implication that CCRVDF originated the four-step process of risk assessment and that this was not already routinely in use by JECFA; the historical interaction between committees such as JECFA and CCRVDF in the evolution of safety factors; the nature of the interaction between CCRVDF and JECFA in the development of policy; and the basis for final agreement of the Codex Alimentarius Commission's position on risk assessment policy.

## 2.5 No-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL): Use in JECFA assessments

The current Committee noted that the sixty-eighth JECFA (additives and contaminants) (Annex 1, reference *187*) had reconsidered the use of the terms no-observed-effect level (NOEL), no-observed-adverse-effect level (NOAEL) and the related terms lowest-observed-effect level (LOEL) and lowest-observed-adverse-effect level (LOAEL) in evaluations of the safety of food additives and contaminants. Taking into account common practice in other risk assessment bodies, the current Committee decided to harmonize with the sixty-eighth JECFA and agreed to differentiate between the terms NOAEL and NOEL for the evaluation of veterinary drugs in food. The following definitions, based on WHO Environmental Health Criteria (EHC), No. 170, *Assessing the human health risk of chemicals: Derivation of guidance values for health-based exposure limits* (*6*), were accepted:

***No-observed-adverse-effect level (NOAEL):*** *greatest concentration or amount of a substance, found by experiment or observation, that causes no detectable adverse alteration of morphology, functional capacity, growth, development, or lifespan of the target organism under defined conditions of exposure....*

***No-observed-effect level (NOEL):*** *greatest concentration or amount of a substance, found by experiment or observation, that causes no alteration of morphology, functional capacity, growth, development, or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.*

The Committee noted that it has, up to now, applied the NOEL according to the EHC 70 (Annex 1, reference *76*) definition. This definition includes the statement that the NOEL "causes no detectable, **usually adverse**, alteration of morphology, functional capacity, growth, development, or lifespan of the target" [emphasis added]. The Committee emphasized that the current decision to accept the definitions of NOEL and NOAEL from EHC 170 does not entail any change in its evaluation practice. It is merely harmonizing the terminology used to differentiate between observed effects and observed adverse effects. Hence, this decision has no impact on any of the previous evaluations made by this Committee.

# 3. Comments on residues of specific veterinary drugs

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

## 3.1 Avilamycin

### *Explanation*

Avilamycin is an antibiotic of the orthosomycin family, produced by the fermentation of *Streptomyces viridochromogenes*. It is a mixture of oligosaccharides of orthosomycins, with a linear heptasaccharide chain linked to a terminal dichloroisoeverninic acid (DIA). Avilamycin blocks the formation of the 70S initiation complexes in bacterial protein synthesis by inhibiting the binding of formylmethionine transfer ribonucleic acid (tRNA) to the 30S or 50S ribosomal subunit. Avilamycin is composed of a mixture of avilamycin A (≥60%), avilamycin B (<18%) and 14 minor factors.

Avilamycin is mainly active against Gram-positive bacteria, including *Bacillus* spp., *Clostridium* spp., *Corynebacterium bovis*, *Enterococcus* spp., *Lactobacillus* spp., *Listeria monocytogenes*, *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus* spp. Avilamycin is intended for use as a veterinary medicine in chickens, turkeys, pigs and rabbits to control bacterial enteric infections. It is intended to be administered at a dose of 100 mg/kg in feed to chickens, turkeys and pigs for 21 days. In rabbits, it is intended to be administered at a dose of 80 mg/kg in feed for 28 days. The typical avilamycin content of the fermentation product is 260 mg activity/g.

Avilamycin has not previously been evaluated by the Committee. The Committee evaluated avilamycin to establish an ADI and to recommend MRLs in relevant species at the request of the 17th Session of CCRVDF (*7*).

### *Toxicological and microbiological evaluation*

#### *Biochemical data*

Avilamycin is poorly absorbed, extensively metabolized and rapidly excreted by pigs, rats and poultry. Orally administered avilamycin is excreted primarily in faeces (over 90%), with less than 8% of the ingested drug eliminated in urine. Metabolites are formed as a result of cleavage of the ortho ester linking the C and D rings of avilamycin. The most abundant metabolite is flambic acid, which represents 40–50% of the total residue in urine and faeces and 15–20% of the residue in liver of pigs. No microbiologically active residues have been detected in tissues following avilamycin administration. There are no pharmacokinetic data available for humans.

#### *Toxicological data*

Various forms of avilamycin (i.e. mycelial, crystalline and granular forms) were tested for toxicity. Doses of all forms of avilamycin in the toxicological studies were reported on the basis of the microbiological activity of avilamycin relative to that of the crystalline form.

The acute oral toxicity of avilamycin in mice and rats is low. The oral median lethal dose ($LD_{50}$) of the dried avilamycin products was higher than 390 or 745 mg avilamycin activity/kg body weight (bw) in both mice and rats.

Groups of mice fed avilamycin at levels of 0, 30, 300 or 3000 mg avilamycin activity/kg in the diet (equivalent to 0, 4.5, 45 and 450 mg avilamycin activity/kg bw per day) continuously for 28 days showed a slight increase in feed intake and body weight of the males at 450 mg avilamycin activity/kg bw per day. No treatment-related mortality or toxicity was observed. The NOAEL was 450 mg avilamycin activity/kg bw per day, the highest dose administered. In another study, mice orally exposed to the mycelial form of avilamycin at 0 or 30 000 mg avilamycin activity/kg in the diet (equivalent to 0 or 4500 mg avilamycin activity/kg bw per day) for 28 days showed no mortality or toxicity. The NOAEL was 4500 mg avilamycin activity/kg bw per day, the only dose administered.

Male and female rats received a dried fermentation product of avilamycin at dietary doses of 0, 4, 6 or 10% (equivalent to 0, 596, 894 or 1490 mg avilamycin activity/kg bw per day) for 2 weeks. There were no toxicologically significant effects on body weight, feed consumption, feed conversion efficiency, haematology, clinical chemistry, organ weights or histopathology. The only treatment-associated finding was brown to black discoloration of the waste trays by urine, although urine was yellow in the bladder or when freshly voided. The NOAEL was 1490 mg avilamycin activity/kg bw per day, the highest dose administered.

In male and female rats fed for 2 weeks with crystalline avilamycin (100% activity) at doses of 0, 3000, 30 000 or 60 000 mg avilamycin activity/kg in the diet (equivalent to 0, 300, 3000 or 6000 mg avilamycin activity/kg bw per day), no toxicologically significant effects were found, other than increased alanine aminotransferase (ALT) activity at 3000 and 6000 mg avilamycin activity/kg bw per day and decreased total bilirubin at all doses of avilamycin compared with the concurrent control, with statistical significance in females. However, the level of total bilirubin in treated groups was in the normal range. The induction of ALT was found only in females and was not accompanied by histopathological damage or changes in liver weight. The NOAEL was 6000 mg avilamycin activity/kg bw per day, the highest dose administered.

In male and female rats fed avilamycin at levels of 0, 30, 300 or 3000 mg avilamycin activity/kg in the diet (equivalent to 0, 3, 30 and 300 mg avilamycin activity/kg bw per day) continuously for 28 days, no mortality or toxicity was observed. Weight gains, haematology, blood chemistry and urinalysis parameters were not affected. The NOAEL was 300 mg avilamycin activity/kg bw per day, the highest dose administered.

In another study, groups of rats fed mycelial avilamycin at 0 or 30 000 mg avilamycin activity/kg in the diet (equivalent to 0 or 3000 mg avilamycin activity/kg bw per day) showed no mortality or toxic signs. Weight gains, haematology, blood chemistry and urinalysis parameters were not affected. The NOAEL was 3000 mg avilamycin activity/kg bw per day, the only dose administered.

In dogs orally administered the dried fermentation product of avilamycin (17.8% activity) at 0, 3.56, 35.6 or 178 mg avilamycin activity/kg bw per day by gelatine capsule for 6 months, no treatment-related mortalities or clinical, haematological, biochemical or pathological signs of toxicity were observed, other than changes in serum ALT. Minor changes of ALT within the historical control range were not considered treatment related. The NOAEL for this study was 178 mg avilamycin activity/kg bw per day, the highest dose administered.

Groups of female and castrated male pigs were fed diets with the mycelial cake form of avilamycin at levels of 0, 30, 300 or 3000 mg avilamycin activity/kg (equivalent to 0, 1.2, 12 and 120 mg avilamycin activity/kg bw per day) for 21 weeks. There were no adverse treatment-related differences in clinical, haematological, biochemical or histopathological findings. The NOAEL was 120 mg avilamycin activity/kg bw per day, the highest dose administered.

Mice receiving avilamycin at concentrations of 0, 30, 300 or 3000 mg avilamycin activity/kg in the diet (equivalent to 0, 4.5, 45 and 450 mg avilamycin

activity/kg bw per day) for 104 weeks showed no treatment-related toxicological or carcinogenic effects. The NOAEL was 450 mg avilamycin activity/kg bw per day, the highest dose administered.

In a carcinogenicity study, rats fed avilamycin at doses of 0, 30, 300 or 3000 mg avilamycin activity/kg diet for 1 week were mated and maintained on their treatments during gestation and lactation. The offspring were fed avilamycin derived from mycelial cake with 7% activity at doses of 0, 30, 300 or 3000 mg avilamycin activity/kg in the diet (equivalent to 0, 1.5, 15 and 150 mg avilamycin activity/kg bw per day) or pure avilamycin at 3000 mg avilamycin activity/kg in the diet (equivalent to 150 mg avilamycin activity/kg bw per day) for approximately 2 years. Clotting times were decreased in males receiving avilamycin derived from mycelial cake at 15 and 150 mg avilamycin activity/kg bw per day on weeks 13, 26, 52 and 78, but values recovered at the last two sampling times (weeks 104 and 112). A non-statistically significant increase in pancreatic exocrine adenomas was found in male rats that were fed avilamycin derived from mycelial cake at 15 and 150 mg/kg bw per day (incidence 2/59 and 4/60, respectively, compared with 0/59 for the control group). A higher, but not statistically significant, incidence of thyroid parafollicular cell carcinoma was also observed in male rats treated with avilamycin derived from mycelial cake at 15 and 150 mg/kg bw per day (incidence 5/59 and 4/60, respectively, compared with 1/59 for the control group). The tumour incidences were within the historical control range. There were no differences in any other toxicological parameters. No neoplastic or non-neoplastic effects of pure avilamycin treatment were reported. Avilamycin was not found to cause cancer in this study, and the NOAEL was 150 mg avilamycin activity/kg bw per day, the highest dose administered.

Assays covering an adequate range of genotoxic end-points were conducted with avilamycin. Avilamycin was not genotoxic in any of the assays evaluated. The Committee concluded that avilamycin is not genotoxic.

A three-generation reproductive toxicity study was performed in rats with dietary avilamycin derived from mycelial cake or pure active ingredient. Rats received avilamycin at doses of 0, 30, 300 or 3000 mg avilamycin activity/kg in the diet (equivalent to 0, 1.5, 15 and 150 mg avilamycin activity/kg bw per day). Animals of each generation were maintained on their respective diets for at least 90 days prior to mating and throughout mating, gestation and lactation. Satellite groups of animals were subjected to gross and organ weight examination at 90 days of treatment. Three or four pregnant females of each generation were sacrificed on day 20 of gestation to examine the effects on fetal development. Resulting litters of each generation were reared to 21 days postpartum and examined morphologically. There were no signs

or mortalities specifically attributable to avilamycin treatment over the three generations. Mating performance, pregnancy rate, duration of gestation and total litter loss were comparable for all dietary concentrations. Macroscopic changes at terminal postmortem examination of mated animals were unrelated to treatment. Absolute and relative liver weights were slightly, but statistically significantly, increased in unmated $F_2$ adult females treated with 15 or 150 mg avilamycin activity/kg bw per day. However, the increase was small and not supported by histopathological findings. The small increase in absolute and relative liver weights was found only in $F_2$ females, not in males and not in other generations. The NOAEL was 150 mg avilamycin activity/kg bw per day, the highest dose administered.

Female pigs receiving avilamycin at 0 or 60 mg avilamycin activity/kg in the diet (equivalent to 0 and 2.4 mg avilamycin activity/kg bw per day) for 21 weeks and then artificially inseminated showed no significant change in growth or reproductive performance. Treatment had no effect on any reproductive indices, including the number and weight of piglets at birth and at weaning. It was concluded that feeding of avilamycin to young pigs did not adversely affect on their subsequent reproductive performance. The NOAEL was 2.4 mg avilamycin activity/kg bw per day, the only dose tested.

Pregnant rats received avilamycin once daily at doses of 0, 500, 1000 or 2000 mg/kg bw (equivalent to 0, 132, 264 and 528 mg avilamycin activity/kg bw per day) by oral gavage during gestation days 6–19. No treatment-related effects were observed on survival, clinical signs or maternal reproductive and fetal morphological development parameters. The NOAEL was 528 mg avilamycin activity/kg bw per day, the highest dose administered.

Rabbits administered a dried fermentation product of avilamycin by oral gavage at daily doses of 0, 250, 716 or 2000 mg/kg bw (equivalent to 0, 44.5, 127.4 and 356 mg avilamycin activity/kg bw per day) during gestation days 6–18 showed no treatment-related effects on pregnancy rate, mean percentage of live fetuses, fetal weights or fetal external anomalies. The NOAEL for maternal toxicity, prenatal toxicity and teratogenicity in rabbits was 356 mg avilamycin activity/kg bw per day, the highest dose administered.

Neurobehavioural effects of avilamycin were evaluated in mice and rabbits after a single administration of oral avilamycin at 0, 1500 or 5000 mg/kg bw. There were no significant changes in end-points of neurobehavioural, neurological or autonomic nervous system toxicity in treated mice or neurobehavioural clinical signs in rabbits at any of the doses tested.

Avilamycin has not been used for therapy in human medicine. There are no data available on the effects of avilamycin on human health.

*Microbiological data*

The Committee evaluated minimum inhibitory concentration (MIC) susceptibility, faecal binding interaction and the biological activity of avilamycin residues and used the microbiological decision tree, adopted during the sixty-sixth JECFA (Annex 1, reference *181*) and compliant with Guideline 36 of the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL36) (*8*), to determine the impact of avilamycin on the human intestinal microbiota.

Avilamycin is microbiologically active mainly against Gram-positive bacteria, including some bacterial genera and species representative of the human intestinal flora. Avilamycin exerts no measurable antibacterial activity against *Escherichia coli* (minimum inhibitory concentration required to inhibit the growth of 50% of organisms [$MIC_{50}$] >128 μg/ml) and relatively poor activity against *Bacteroides fragilis*, other *Bacteroides* spp., *Lactobacillus* spp. and *Bifidobacterium* spp. Avilamycin activity is clearly demonstrable against *Peptostreptococcus* ($MIC_{50}$ = 0.25 μg/ml), *Eubacterium* ($MIC_{50}$ = 0.5 μg/ml) and *Clostridium* spp. ($MIC_{50}$ = 1 μg/ml).

Avilamycin residue is extensively metabolized to products with very low microbiological activity prior to entering the human colon. In addition, no microbiologically active residues are detected in edible tissues from chicken and pigs. Avilamycin binds to human faeces rapidly, extensively and irreversibly. Residues of avilamycin are substantially bound (>95%) to faecal material within the colon, further reducing their microbiological activity. Therefore, avilamycin residues are unlikely to disrupt the colonization barrier of the human gastrointestinal tract.

Although avilamycin selects for resistance in the native enterococcal population of pigs, no resistant isolates were detected beyond 1 week post-treatment. This suggests that resistant isolates are unable to persist once selective pressure is removed and are outcompeted by sensitive microflora. The only drug that shows cross-resistance to avilamycin is the structurally related antibiotic evernimicin, developed for, but not introduced into, human medicine. The intrinsic resistance exhibited by most Gram-negative bacteria to avilamycin is attributed to their lower membrane permeability, which limits the accessibility of the target by avilamycin. Resistance to avilamycin in Gram-positive bacteria occurs exclusively by modification of the drug target; in other words, alterations in the antibiotic-binding site of the ribosome decrease the binding affinity of the drug. This is incomplete cross-resistance, in that enterococci resistant to avilamycin exert only decreased susceptibility and not complete resistance to evernimicin. In addition, the avilamycin class of antimicrobial compounds is not used in human medicine. Considering the existing information, the development of resistance to avilamycin and

cross-resistance to a number of commonly used antimicrobials in veterinary and human medicine is not likely.

Based on the available data, a microbiological ADI for avilamycin is not needed.

*Evaluation*

The most relevant animal studies for evaluation of the safety of residues of avilamycin in food are the 2-year toxicity study and the multigeneration reproductive study in rats. No significant adverse effects were observed following avilamycin administration, and the NOAEL was 150 mg avilamycin activity/kg bw per day, the highest dose administered in both studies. A toxicological ADI of 0–2 mg avilamycin activity/kg bw per day is established, using a safety factor of 100 and rounding to one significant figure.

A toxicological monograph was prepared.

### *Residue evaluation*

*Data on pharmacokinetics and metabolism*

The oral bioavailability of avilamycin in pigs, chickens and rats is low. Complete pharmacokinetic data are not available.

Avilamycin is rapidly excreted in faeces when administered orally to pigs. Within the first 9 days following treatment with radiolabelled drug, 92–93.4% was excreted in the faeces and 4.5–8% in the urine. Similar results were found in chickens, for which an average of 90% of the radioactivity was found in excreta within the first 6 days.

Pharmacokinetic data in turkeys and rabbits are not available. Pharmacokinetic data in rats, pigs and chickens are highly consistent. Owing to similarity of species, pharmacokinetic data in chickens may be applied to turkeys.

The metabolite pattern in urine and faeces of treated pigs was essentially the same as the pattern for rats. In both species, there were three faecal metabolites that were derived from the oligosaccharide and eurekanate portion of avilamycin. The most abundant metabolite was flambic acid. This metabolite has been previously identified as flambalactone. Flambic acid and flambalactone were found to be interconvertible and represent 30–60% of the acidic portion of the faeces. Parent avilamycin constituted less than 10% of the faecal radioactivity in pigs.

The metabolites in liver of rats and pigs treated with radiolabelled avilamycin were essentially the same. Flambic acid was the most abundant metabolite in both species (15–20% of liver residue). Avilamycin residues in rat and pig

livers were less than 0.05 mg/kg. The pattern of minor metabolites was similar, but none of the minor metabolites were sufficiently abundant for identification. Characterization of fat samples from pigs demonstrated that essentially all of the residues in fat were due to the incorporation of radioactivity into the endogenous fatty acids.

No metabolism data are available for chickens, turkeys or rabbits.

*Residue data*

As avilamycin is extensively metabolized, the position of the radiolabel is important for the correct interpretation of the total radioactive tissue data.

DIA is a moiety present in avilamycin, flambic acid and other possible metabolites, which can be released by chemical hydrolysis. Advantage is taken of this hydrolysis in the analytical procedure to measure residues derived from avilamycin. DIA is proposed as the marker residue for avilamycin.

Three Good Laboratory Practice (GLP)-compliant radiolabelled residue studies in pigs were submitted. Two of them used avilamycin labelled in the DIA moiety [DIA-$^{14}$C], and the third used uniformly labelled [U-$^{14}$C] avilamycin. One GLP-compliant study in chickens using [DIA-$^{14}$C]avilamycin was submitted. In all studies, animals were slaughtered at a practical zero-time withdrawal of 6 h after the final feeding of medicated ration.

Five crossbred pigs (three males and two females), weighing approximately 46 kg each, were fed at 12-h intervals for 7 days with a ration containing 76.19 mg [DIA-$^{14}$C]avilamycin/kg feed (equivalent to 80 mg activity/kg and equal to 9.2–12.1 mg/kg bw per day). One male was sacrificed at the final feeding of medicated ration. The remaining animals were then fed non-medicated ration at 12-h intervals for either 3 or 5 days, and one male and one female were sacrificed at the end of each time period. Concentrations of avilamycin-related radioactivity in liver and muscle declined to non-detectable levels (limit of detection [LOD] = 0.024 and 0.033 mg/kg, respectively) within 3 days after the termination of dosing, whereas concentrations in kidney declined to near non-detectable levels within 5 days after the termination of dosing (LOD = 0.025 mg/kg). Radioactivity in fat showed a much slower rate of decline due to [$^{14}$C]avilamycin being incorporated into the fatty acid fraction.

In a similar study using the same radiolabelled compound and dose, nine crossbred pigs weighing approximately 44 kg each were fed at 12-h intervals for 4, 7 or 10 days. After 10 days of dosing, total radioactive residues in liver, muscle, fat and kidney, expressed as avilamycin equivalents, were 0.22, 0.016, 0.12 and 0.10 mg/kg, respectively. Steady-state concentrations of radioactivity were attained in muscle, liver and kidney within 4 days after the

initiation of dosing. A steady-state concentration in fat was not attained during this study; residues were 0.121 mg/kg at 10 days.

No residues of parent avilamycin were detected in pig kidney or fat analysed by thin-layer chromatography coupled with bioautography after 10 days of treatment, and only traces were detected in liver. Muscle was not assayed owing to very low amounts of radioactivity (limit of quantification [LOQ] <0.05 mg/kg). Approximately 50% of total radiolabelled residues in liver were DIA-related residues analysed by a gas chromatographic (GC) method. DIA-related residues were detected in kidney, but were less than the LOQ (<0.1 mg/kg). No DIA residues were observed in fat (<0.1 mg/kg).

A steady-state tissue residue study was conducted in pigs dosed with [U-$^{14}$C]-avilamycin. Six crossbred pigs weighing approximately 44 kg each were fed at 12-h intervals for either 10 or 14 days with a ration containing a nominal concentration of 60 mg [$^{14}$C]avilamycin/kg feed (equivalent to 60 mg activity/kg and equal to 7.2–9.6 mg/kg bw per day). After 10 days of dosing, total radioactive residues expressed as avilamycin equivalents in liver, fat, muscle and kidney were 0.554, 0.261, 0.093 and 0.321 mg/kg, respectively. There was no statistical difference in total radioactive residues in muscle, liver or kidney at 10- or 14-day dosing times, indicating that steady state was achieved. Only the radioactive residues in fat were significantly different between 10 and 14 days. The GC analysis showed that extractable liver radioactivity consisted of several minor metabolites (<0.1 mg/kg). Flambic acid was present at concentrations up to 0.04 mg/kg. Parent [$^{14}$C]avilamycin concentrations were less than 0.01–0.02 mg/kg.

Seven-week-old broiler-type chickens (six males and six females) were fed a standard broiler finishing ration containing 14.16 mg [DIA-$^{14}$C]avilamycin/kg feed (equivalent to 15 mg activity/kg and equal to 3 mg/kg bw per day) for 4, 7 or 10 days. After 10 days of dosing, the total radioactive residues, expressed as avilamycin equivalents, in skin, liver and fat were 0.020, 0.022 and 0.026 mg/kg, respectively. Muscle and kidney samples contained no detectable radiolabel residues. Steady-state concentrations of radioactivity were attained in all tissues within 4–7 days after the initiation of dosing.

DIA has been proposed as the marker residue for avilamycin, as previously mentioned.

The only tissue with measurable residues at 6-h withdrawal times is liver, and thus it is the only possible target tissue.

No radiolabelled depletion studies on turkeys or rabbits are available.

One GLP-compliant non-radiolabelled residue depletion study was provided for pigs, chickens, turkeys and rabbits.

Crossbred commercial pigs (six males and six females) weighing 9–15 kg each were fed, ad libitum, a commercial diet containing avilamycin at a nominal concentration of 150 mg/kg feed for 21 consecutive days (equal to 9–12 mg/kg bw per day). At the end of the 21-day exposure period, animals were euthanized at withdrawal intervals of 0, 6 and 24 h. Samples of liver, kidneys, muscle and skin with fat were collected. Avilamycin residues were analysed as DIA equivalents by a liquid chromatography/tandem mass spectrometry (LC-MS/MS) validated method and also by a microbiological assay. The results showed that the DIA moiety of avilamycin was quantifiable in pig liver at 0 and 6 h withdrawal, declining by more than half in 6 h. After 24 h, the residues were below 28 μg avilamycin equivalents/kg tissue (lowest point on the standard curve). DIA residues were detected, but not quantifiable, in kidney at 0 and 6 h withdrawal and were not detected after 24 h. No residues were detected in muscle or skin/fat samples at any time. No antimicrobial activity was detected in any tissue by an inhibition assay using *Micrococcus luteus* (LOD = 5 μg/kg). Therefore, DIA residues detected in liver and kidney were due to microbiologically inactive metabolites of the drug.

In several non-GLP-compliant studies submitted, increasing the dose of non-radiolabelled avilamycin added to pig rations to 200 mg/kg did not result in detectable levels of microbiologically active residues in the tissues of pigs analysed by an inhibition assay using *Micrococcus flavus* (LOD = 0.05 mg/kg).

Broiler chickens (15 males and 15 females) approximately 2 weeks old weighing 339–541 g were fed, ad libitum, a commercial diet containing avilamycin at a nominal concentration of 150 mg/kg feed for 21 consecutive days (equal to 30 mg/kg bw per day). At the end of the 21-day exposure period, animals were sacrificed at withdrawal intervals of 0, 6 and 24 h. Samples of liver, kidneys, muscle and skin/fat (subcutaneous) were collected. The results showed that DIA was quantifiable in chicken liver at zero-time withdrawal and declined to below or near 28 ng avilamycin equivalents/kg tissue within 6 h. After 24 h, the residues were below 28 μg avilamycin equivalents/kg tissue (lowest point on the standard curve). DIA residues were detected, but not quantifiable, in kidney and skin/fat at 0 and 6 h withdrawal and were not detected after 24 h. Skin/fat and kidney samples did not have detectable residues after 6 h withdrawal. No DIA residues were detected in muscle samples at any time. No antimicrobial activity was detected in any other tissue by an inhibition assay using *Micrococcus luteus* (LOD = 5 μg/kg).

In several non-GLP-compliant studies submitted, increasing the dose of non-radiolabelled avilamycin added to chicken rations to 200 mg/kg did not result in detectable levels of microbiologically active residues in the tissues of chickens analysed by an inhibition assay using *Micrococcus flavus* (LOD = 0.05 μg/g).

Turkeys (three males and two females) approximately 8 weeks old weighing 2.9–5.2 kg each were fed, ad libitum, a commercial diet containing avilamycin at a nominal concentration of 150 mg/kg feed (equal to 30 mg/kg bw per day) for 7 consecutive days. At the end of the 7-day exposure period, animals were euthanized at zero withdrawal time. Samples of liver, kidneys, muscle and skin with fat were collected and analysed using the routine analytical method (LC-MS/MS for DIA), which was validated for chicken tissues and whose applicability was demonstrated for turkey tissues. The residues at zero withdrawal time were very low in liver (67.6–195 µg avilamycin equivalents/kg tissue) and skin/fat (37.3–105 µg avilamycin equivalents/kg tissue) and below 28 µg avilamycin equivalents/kg tissue (lowest point on the standard curve) in muscle and kidney. Antimicrobial activity was not found in liver, kidney or muscle samples.

Rabbits (three males and two females) approximately 7 weeks old weighing 1.06–1.46 kg each were fed, ad libitum, a commercial diet containing avilamycin at a nominal concentration of 125 mg/kg feed (equal to 7.7 mg/kg bw per day) for 7 consecutive days. At the end of the 7-day exposure period, animals were euthanized at zero withdrawal time. Samples of liver, kidney, muscle and fat were collected and analysed using the LC-MS/MS method for DIA developed for pig tissues and whose applicability was demonstrated for rabbit tissues. The residues at zero withdrawal time were very low in liver and kidney (93–145 and 228–352 µg avilamycin equivalents/kg tissue, respectively) and below 28 µg avilamycin equivalents/kg tissue (lowest point on the standard curve) in muscle and fat. Antimicrobial activity was not found in liver, fat, kidney or muscle samples.

For considering MRL values, it is necessary to estimate a ratio of marker residue (DIA) to total residues. For pig liver, available data indicate a ratio of 0.5. For the other pig tissues and the other species, this ratio could not be established on an experimental basis owing to the low or non-detectable residue concentrations. A conservative ratio of 0.1 was considered appropriate for recommending MRLs in other species and tissues.

### *Analytical methods*

Analytical methods for the determination of avilamycin in pig and chicken tissues (muscle, skin/fat, liver and kidney) have been developed. The applicability of these methods to turkey and rabbit tissues was demonstrated. Avilamycin and/or its metabolites containing DIA were extracted from homogenized tissues and hydrolysed to release DIA. The DIA concentration was measured by gradient high-performance liquid chromatography (HPLC) using negative-ion electrospray ionization mass spectrometry (ESI-MS) and converted to avilamycin equivalents by multiplying the determined DIA concentration by the molar ratio of avilamycin to DIA (5.6).

The method was validated by the sponsor at three concentrations for all tissues in all species. The sponsor adopted the LOQs as the minimum concentrations in fortified samples that were shown to satisfy the criteria for recovery and precision.

The Committee reconsidered the data supplied and calculated the LOQs considering the representative chromatograms of the extracted fortified samples for pigs and chickens and using the criterion of signal to noise ratio equal to 10. The LOQs expressed as DIA determined for pigs are 24, 22.4, 3.3 and 10 µg/kg for muscle, skin/fat, kidney and liver, respectively. The LOQs expressed as DIA determined for chickens are 18.8, 18.7, 22.4 and 30.4 µg/kg for muscle, skin/fat, kidney and liver, respectively.

*Maximum residue limits*

The following data have been taken into account in recommending MRLs for avilamycin:

- A toxicological ADI of 0–2 mg/kg bw was established, which is equivalent to a daily intake of 0–120 mg for a 60-kg person.
- Avilamycin is poorly absorbed and extensively metabolized.
- Metabolism studies are available in rats and pigs. No metabolism data are available for chickens, turkeys or rabbits.
- DIA was selected as the marker residue.
- Residue concentrations of the marker residue were not quantifiable or detected in muscle, skin/fat or kidney in pigs and chickens at a withdrawal time of 0 h or greater. Low residue concentrations were present in liver of all species studied in the first hours post-treatment, but were not quantifiable or detected after 24 h withdrawal.
- For pig liver, a ratio of marker residue to total residue of 0.5 has been established. For the other pig tissues and the other species, the ratio could not be established on an experimental basis owing to the low or non-detectable residue concentrations. A conservative ratio of 0.1 was adopted.
- No microbiologically active residues were detected in edible tissues of pigs, chickens, turkeys or rabbits.
- A validated routine analytical method for the determination of the marker residue in edible tissues of pigs, chickens, turkeys and rabbits is available.

— A conservative estimate of approximately 10 × LOQ expressed as DIA was used to recommend MRLs for chickens. Pig MRLs have been harmonized with chicken MRLs. Chicken MRLs may be extended to turkeys based on similarity between the species. For rabbits, MRLs may be harmonized with those for minor species.

The recommended MRLs are expressed as the marker residue DIA. Rounded values are 200 μg/kg for muscle, 200 μg/kg for skin/fat, 200 μg/kg for kidney and 300 μg/kg for liver for pigs, chickens, turkeys and rabbits.

The EDI was not estimated because of insufficient data points with which to calculate the median values of residues (low quantities of residues or absence of quantifiable residues).

Using the model diet and avilamycin equivalents, the recommended MRLs would result in a daily intake of 5.3 mg of avilamycin, which represents approximately 4% of the upper bound of the ADI.

A residue monograph was prepared.

## 3.2 Dexamethasone

### *Explanation*

Dexamethasone is a fluorinated glucocorticosteroid and a potent anti-inflammatory agent used frequently for treatment of inflammatory processes and primary ketosis in domestic food-producing animals. Dexamethasone was evaluated at the forty-second and forty-third meetings of the Committee (Annex 1, references *110* and *113*). At the forty-second meeting, the Committee established an ADI of 0–0.015 μg/kg bw for dexamethasone and recommended the following temporary MRLs: 0.5 μg/kg for muscle, 2.5 μg/kg for liver and 0.5 μg/kg for kidney, expressed as parent drug, in cattle and pigs; and 0.3 μg/l for cows' milk, expressed as parent drug. At its forty-second meeting, the Committee noted that dexamethasone undergoes extensive metabolism. However, it also noted that the metabolites did not exhibit any biological activity and consequently proposed dexamethasone as the marker residue. The MRLs were designated as temporary because an adequate method to determine compliance with the MRL was not available. At the forty-third meeting, the same temporary MRLs were recommended for horses as were recommended at the forty-second meeting for cattle and pigs. At the fiftieth meeting (Annex 1, reference *134*), the Committee reviewed documentation on an HPLC-MS method in tissue and milk. Selectivity was not judged adequate because of the partial coelution of betamethasone (16β-isomer); unambiguous identification of dexamethasone was considered to be difficult. Large variation in detector response was reported to occur during

analysis. Calculation of quantitative results in incurred samples may not be accurate, because nonspecific interferences are encountered occasionally. The Committee concluded that the method did not meet the required performance criteria for identification and quantification of incurred residues in tissues and milk.

Two sponsors provided three methods for muscle/kidney, liver and milk.

At the 17th Session of CCRVDF (*7*), a request for MRL recommendations in cattle (tissues, milk), horses (tissues) and pigs (tissues) was raised.

### *Analytical methods*

LC methods based on ultraviolet (UV) detection were considered unsuitable for residue analysis at sub-microgram per kilogram (or sub-microgram per litre) concentrations. Methods based on GC-MS (negative chemical ionization) after dexamethasone oxidation are no longer used. LC electrospray (positive or, better, negative mode) MS methods are preferred because they provide better sensitivity and specificity. The analytical method for muscle and kidney from cattle consists of one common procedure using LC-MS/MS (ESI+); sample preparation was performed using two solid-phase extraction (SPE) purification steps. The chromatographic method involves gradient elution using a reversed-phase column. No mention of the use of any internal standard is provided for muscle and kidney. The analytical method for liver and milk from cattle consists of two different procedures using LC-MS/MS (ESI); sample preparation was performed using one SPE purification step. The chromatographic method involves isocratic elution using a reversed-phase column. A chemical analogue (9α-fluoroprednisolone) was utilized for dexamethasone identification and quantification.

The validation of the method for muscle and kidney has been conducted with a target residue level set at 1 μg/kg, whereas the MRL recommended previously by the Committee was 0.5 μg/kg. The validation of the method for liver tissue has been performed for a target residue level of 2 μg/kg, whereas the previous MRL in liver was 2.5 μg/kg. The validation of the method for milk was performed at a target residue level of 0.3 μg/l, equal to the previously recommended MRL for milk.

Selectivity has been proved as fitting with the needs; in particular, the efficient chromatographic separation of betamethasone and dexamethasone was made possible. The LOQ (defined as the lowest validated level) and limit of identification were 0.4 μg/kg for both muscle and kidney. The LOQs were 0.15 μg/l for milk and 1 μg/kg for liver. The limit of decision (CCα,[1] risk

[1] CCα means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

α = 5%) and detection capability (CCβ,[2] risk β = 5%) were 1.2 μg/kg and 1.5 μg/kg, respectively, for muscle and kidney. These performances fit the expectations of a MRL method at 1.0 μg/kg, but are insufficient considering the MRL as recommended by the Committee at its forty-second and forty-third meetings. Limits of decision and detection capabilities were 0.45 μg/l and 0.57 μg/l, respectively, for milk and 2.9 μg/kg and 3.7 μg/kg, respectively, for liver. These performances have been calculated in reproducibility conditions, which can be considered as conservative. Any laboratory implementing the methods will characterize an "in-house" decision limit in repeatability conditions. The CCα would then be significantly closer to the MRL. The same applies to the CCβ. In summary, the performances of the methods are fulfilling the minimum performance criteria corresponding to dexamethasone residues in milk and liver at the MRL as recommended by the Committee at its forty-second and forty-third meetings.

### *Maximum residue limits*

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

- The established ADI is 0–0.015 μg/kg bw, equivalent to 0–0.9 μg for a 60-kg person.
- The marker residue is dexamethasone.
- The appropriate target tissues are liver or kidney and milk.
- A suitable validated routine method was available for monitoring dexamethasone in bovine milk and liver at 0.3 μg/l and 2.0 μg/kg, respectively.
- A suitable validated routine method was available for monitoring dexamethasone in bovine muscle and kidney at 1.0 μg/kg, but not at 0.5 μg/kg.
- No validated method for horses and pigs was provided or could be found, but the method provided for cattle tissue is adequate to be extended to pig and horse tissues.
- The recommended MRLs are based on the performances of the analytical methods (2 × LOQ).

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, pigs and horses, expressed as the

[2] CCβ means the smallest content of the substance that may detected, identified and/or quantified in a sample with an error probability of β.

marker residue dexamethasone: muscle/kidney, 1.0 μg/kg; liver, 2.0 μg/kg; and cows' milk, 0.3 μg/l. Based on these values for the MRLs, the maximum theoretical intake would be 1 μg/day per person. This would be compatible with a maximum ADI of 0.9 μg for a 60-kg person. The Committee noted that at its forty-second meeting, it was concluded that dexamethasone is rapidly eliminated from muscle and milk and that the probability of exposure to residues from these tissues is low.

An addendum to the residue monograph on dexamethasone was prepared.

## 3.3 Malachite green

### *Explanation*

Malachite green (MG) is an *N*-methylated triphenylmethane dye that is used mainly industrially for leather, wool, cotton, silk, jute, paper and certain fibres. For such purposes, large quantities of extremely variable composition have been produced. About 10–15% of all dyes are lost directly to wastewater in the dyeing process.

The chemical has been used routinely in some countries in aquaculture since the early 1930s and is considered by many in the fish industry to be an effective antifungal and antiprotozoal agent in fish, fish eggs and crayfish. It is currently registered in some countries for use as a veterinary drug in ornamental fish, to which it is applied as a topical antiseptic or to treat parasites, fungal infections and bacterial infections in fish and fish eggs. It is not permitted in aquaculture of fish destined for human consumption. Reported types of treatment of fish include dip treatment, flush treatment, sustained culture treatment and application in feed. Extremely wide ranges of concentrations and exposure times have been used.

MG in water originating from contamination as a result of its industrial applications or from its illegal use in aquaculture is efficiently taken up from the water by fish and distributed to all tissues. MG is metabolically reduced by fish to the persistent colourless metabolite, leucomalachite green (LMG), and possibly other, as yet unidentified, degradation products. The rate of excretion of MG (as LMG) from fish is dependent on the fat content of the fish, with more LMG being retained in fatty fish than in lean fish. Therefore, when fish that have been exposed to MG reach the consumer, the amount of LMG present in the fish is expected to be higher than that of MG, because of its longer elimination half-life.

MG has not previously been evaluated by the Committee. It was placed on the agenda of the current meeting at the request of the 17th Session of CCRVDF (*7*), which requested JECFA to consider a literature review and

advise if this substance could be supported for use in food-producing animals (as the available data were probably not sufficient to derive an ADI and MRLs).

Two risk assessments were provided by national authorities. In addition, a comprehensive literature search was performed.

### *Toxicological evaluation*

#### *Biochemical data*

There is only limited information available on the absorption, distribution, metabolism and excretion of MG in mammalian species, including humans. In male and female rats given an oral dose of 2 mg $^{14}$C-labelled MG/kg bw, more than 95% of the radioactivity was excreted within 7 days, with more than 80% appearing in the faeces. No attempt was made to identify any metabolites. In vitro studies have shown that MG is readily converted into LMG under anaerobic conditions by a multitude of bacterial species present in the intestinal microflora from mice, rats, rhesus monkeys and humans. Therefore, MG that is ingested can be converted to LMG by the intestinal microflora.

In short-term feeding studies, it was shown that MG is sequentially *N*-demethylated to secondary and primary aromatic amines in rats and mice both before and after reduction to LMG. Analysis of liver extracts from rats treated with MG by HPLC coupled to atmospheric pressure chemical ionization mass spectrometry (APCI-MS) identified MG, its mono-, di-, tri- and tetradesmethyl derivatives and MG *N*-oxide. A small, but measurable, amount of LMG was also detected. Concentrations of MG and metabolites increased with increasing dose. Similarly, LMG, demethylated derivatives, MG *N*-oxide and demethylated *N*-oxide derivatives were detected in liver extracts from rats treated with LMG. A small, but measurable, amount of MG was also detected.

MG, in contrast to LMG, is highly cytotoxic to bacteria and mammalian cells in vitro. Studies using mammalian cells showed that MG treatment resulted in induction of the cytochrome P450 (CYP) mono-oxygenase system, lipid peroxidation and catalase activity in a concentration-dependent manner.

#### *Toxicological data*

*Malachite green.* In short-term (28 days) feeding studies in rats with doses ranging from 1 to 120 mg MG/kg bw per day, MG produced haematological changes (decreased erythrocyte count and haemoglobin and haematocrit values) and increased the relative liver weight in both sexes. This effect was

accompanied by increased levels of gamma glutamyl transferase activity in the blood. Vacuolization of hepatocytes, primarily midzonal and centrilobular, was observed at the highest dose level tested. The NOAEL was 10 mg MG/kg bw per day based on haematological changes and effects on the liver, both of which were seen at 30 mg MG/kg bw per day and higher dose levels.

When rats were dosed with 120 mg MG/kg bw per day for 21 days, there was a significant increase in the triiodothyronine ($T_3$) levels and a significant decrease in the thyroxine ($T_4$) levels in the females. Thyroid-stimulating hormone (TSH) was not affected, and no effects were seen in the males.

In a study using male Wistar rats pretreated for 4 weeks with *N*-nitrosodiethylamine (DEN), it was shown that administration of MG in the drinking-water (resulting in daily doses ranging from 1.88 to 7.5 mg/kg bw per day) for 7 months dose-dependently enhanced the DEN-induced liver carcinogenicity to a significant extent. Increases in the relative liver weights and biochemical markers for hepatic preneoplastic lesions and cell cycle regulatory proteins were noted. A subsequent biochemical study confirmed that a number of markers for liver cell proliferation and cell cycle regulation were altered. The results provided evidence for dysregulation of checkpoints of the cell cycle as a possible mechanism during promotion of hepatic preneoplastic lesions by MG.

In a teratogenicity study in rabbits, MG (0, 5, 10 or 20 mg MG/kg bw per day from gestation days 6 to 18) was reported to produce a dose-related decrease in weight gain or marked weight loss in the dams and increased incidences of fetal anomalies (gross, visceral and skeletal) in all treated groups. A NOAEL could not be established. The lack of consistent dose–response relationships in most of the effects seen in the pups is noteworthy, but might be due to the very narrow dose range studied. The study was inadequately conducted and reported, and additional studies would be needed to properly address the potential of MG to produce reproductive and developmental toxicity.

Administration of MG and its major metabolite LMG at doses up to 60 and 58 mg/kg bw per day, respectively, for 28 days gave rise to a single liver deoxyribonucleic acid (DNA) adduct species (or co-eluting adducts) in male Fischer 344 (F344) rats. Females were not studied. The adduct level increased significantly as a function of the dose and did not differ between groups administered equimolar doses of MG and LMG. In female B6C3F1 mice, doses up to 90 and 87 mg/kg bw per day for 28 days for MG and LMG, respectively, also gave rise to a single DNA adduct species (or co-eluting adducts), the level of which increased as a function of the dose for MG, but only minimally and borderline for LMG. Thus, in the female mouse, MG produced much higher adduct levels than LMG at equimolar doses. In contrast, in the female

Big Blue F344 transgenic rat, LMG at dose levels up to 54.3 mg/kg bw per day for up to 32 weeks gave rise to a single DNA adduct species, the level of which increased linearly with the dose.

MG did not produce mutations in the Ames test using *Salmonella typhimurium* strains or in Chinese hamster ovary cells (CHO-K1) and produced DNA damage in the comet assay in CHO-K1 cells only at cytotoxic concentrations. It did not produce a clastogenic effect in the in vivo mouse bone marrow micronucleus test and did not increase the occurrence of recessive mutations when tested in the mammalian spot test in mice. In addition, MG did not induce micronuclei in erythrocytes, mutations in lymphocytes or (in contrast to LMG) *cII* mutations in liver cells of female Big Blue B6C3F1 transgenic mice administered 67.5 mg MG/kg bw per day for 16 weeks.

It is concluded that although MG DNA adducts have been identified in the liver of male rats and female mice, the weight of evidence indicates that MG has no genotoxic potential in conventional in vitro and in vivo assays and did not produce mutations in the liver of transgenic female mice at the dose levels applied.

MG was tested for carcinogenicity in female F344 rats fed diets corresponding to daily intakes of 0, 7, 21 or 43 mg/kg bw per day for 2 years. A reduced body weight gain was observed at the two highest dose levels. There was a trend in increased occurrence of thyroid gland follicular cell adenoma or carcinoma, being statistically significant only at the middle dose level. Hepatocellular adenomas were minimally (but not statistically significantly) increased at the two highest dose levels, and there was a non-significant trend in the incidence of mammary gland carcinomas in the treated animals (see Table 2). The NOAEL for non-cancer effects was 7 mg MG/kg bw per day based on the reduced body weight gain at the two highest dose levels.

MG was also tested for carcinogenicity in female mice. No increases in tumour incidences were seen in female mice fed diets corresponding to daily intakes of 15, 33 or 67 mg MG/kg bw per day for 104 weeks. No effects on mortality or body weight gain were observed.

*Leucomalachite green.* LMG was tested in short-term (28 days) feeding studies in female B6C3F1 mice at doses ranging from 43.5 to 174 mg/kg bw per day and in male F344 rats at doses ranging from 29 to 116 mg/kg bw per day. The NOAEL in female mice was 43.5 mg LMG/kg bw per day based on reduced body weights seen at higher dose levels. All females at the highest dose level had scattered dead or degenerate cells in the transitional epithelium of the urinary bladder. When LMG was tested in male rats, increased relative liver weights were reported for all dose groups, and the animals in the two highest dose groups had significantly lower body weights. At the highest dose level, gamma glutamyl transferase activity and phosphorus levels were

significantly increased, whereas the erythrocyte count, haemoglobin and haematocrit levels were significantly decreased. Vacuolization of hepatocytes, primarily midzonal and centrilobular, was observed at all dose levels. Apoptotic follicular epithelial cells in the thyroid gland were seen in some rats at the two highest dose levels. A NOAEL could not be established in this study.

Table 2
**Tumours in F344 female rats administered MG (0, 7, 21 or 43 mg/kg bw per day) for 2 years**

| Tumour type | Tissue toxicity | DNA adducts | Genotoxicity/ mutagenicity | Other effects |
|---|---|---|---|---|
| Liver adenoma<br>1/48, 1/48, 3/48, 4/48<br>Historical control: 1 adenoma in 6 studies | Eosinophilic foci<br>5/48, 10/48, 13/48, 14/48<br>Necrosis at highest dose<br>Increased relative liver weight | A single liver DNA adduct species in male rats and female mice | Negative in conventional tests and transgenic female mice<br>Positive in comet assay in CHO cells at cytotoxic concentrations | Promoter of DEN-initiated liver tumours<br>Decreased body weight |
| Thyroid adenoma and carcinoma<br>0/46, 0/48, 3/47, 2/46<br>Historical control: NCTR 1.4% and NTP 0.9% | Cystic follicles<br>0/46, 1/48, 1/47, 3/46<br>Thyroid weight was not affected | | | 120 mg/kg bw per day:<br>$T_3 \uparrow$, $T_4 \downarrow$<br>Decreased body weight |
| Mammary carcinoma<br>2/48, 2/48, 1/48, 5/48<br>Historical control 0.7% | | | | Decreased body weight |

NCTR, National Center for Toxicological Research (USA); NTP, National Toxicology Program (USA).

In male rats given 116 mg LMG/kg bw per day for 21 days, there was a significant increase in the serum TSH levels and a significant decrease in the $T_4$ levels.

LMG did not induce mutations in any of the tested *S. typhimurium* strains and in Chinese hamster ovary cells (CHO-K1) and was negative for DNA damage

in the comet assay in CHO-K1 cells. It did not produce any significant increase in the *lacI* mutation frequencies or changes in the mutation spectrum of *lacI* mutants in female Big Blue rats administered dietary doses up to 54.3 mg LMG/kg bw per day for up to 32 weeks. In addition, no effect was observed in the liver *cII* mutation frequency, the *Hprt* lymphocyte mutant assay or the bone marrow micronucleus assay in these rats.

LMG did not induce micronuclei in erythrocytes or mutations in lymphocytes of female Big Blue B6C3F1 transgenic mice administered 0, 30.6 or 61.2 mg LMG/kg bw per day for 16 weeks. However, LMG induced *cII* mutations with an increased frequency of guanine to thymine (G → T) and adenine to thymine (A → T) transversions in the liver cells of the female mice at the highest dose level tested (61.2 mg LMG/kg bw per day).

LMG was tested for carcinogenicity in male and female rats fed diets corresponding to dose levels of 0, 5, 15 or 30 mg/kg bw per day or 0, 6, 17 or 35 mg/kg bw per day, respectively, for 2 years. Mortality was not affected. Reduced body weight gains were observed at the two highest dose levels in both sexes. A low, not statistically significant increase in the incidence of thyroid gland follicular cell adenoma or carcinoma (2–7%) was seen in both sexes. There was no significant effect on the incidence of hepatocellular adenomas in the rats fed LMG. The mammary gland adenoma or carcinoma incidence in female rats was 0/48, 2/48, 3/48 and 4/48 at 0, 6, 17 and 35 mg/kg bw per day, respectively. The NOAEL for non-cancer effects was 5 mg/kg bw per day based on the reduced body weight gains seen at the higher dose levels.

In female mice fed diets corresponding to intakes of 0, 15, 31 and 63 mg LMG/kg bw per day for 104 weeks, the only finding was a dose-related trend in increased incidence of hepatocellular adenomas or carcinomas (3/47, 6/48, 6/47 and 11/47, respectively), with the incidence being statistically significant in the highest dose group (Table 3).

Table 3
**Tumours in B6C3F1 female mice administered LMG (0, 15, 31 or 63 mg/kg bw per day) for 2 years**

| Tumour type | Tissue toxicity | DNA adducts | Genotoxicity/mutagenicity |
|---|---|---|---|
| Liver adenoma and carcinoma<br>3/47, 6/48, 6/47, 11/47 | No toxicity reported | Liver DNA adduct species only borderline in female mice<br>A single liver DNA adduct species seen in male and female rats | Negative in conventional in vitro and in vivo tests and in female transgenic rats<br>Induced *cII* mutations in transgenic female mice |

*Consideration of mode of action for MG and LMG.* The incidences of tumours in female rats administered MG for 2 years and of tumours in female mice administered LMG for 2 years are shown in Table 2 and Table 3, respectively, together with findings related to the possible mode of action.

Both MG and LMG caused a weak, statistically non-significant increase in the occurrence of thyroid gland follicular cell adenoma and carcinoma in rats. Short-term (28 days) studies showed an increase in $T_3$ and a decrease in $T_4$ after MG treatment in female rats, and LMG treatment resulted in an increase in TSH and a decrease in $T_4$ levels. Mechanistic studies in vitro with LMG showed an inhibition in thyroid peroxidase–catalysed tyrosine iodination. This would support the view that chronic exposure to MG and LMG could result in thyroid gland follicular cell tumours through a hormonal, thresholded mechanism.

The tumour data supporting a treatment-related effect for MG and LMG in the induction of liver carcinogenicity in rats are not strong, with only the female rats treated with the two highest doses of MG having a minimally increased (not statistically significant) occurrence of hepatocellular adenomas. Although a dose-dependent formation of a DNA adduct species was demonstrated in the livers of F344 and Big Blue rats administered MG or LMG in the diet, analyses of liver *lacI* and *cII* mutants revealed that the mutant frequencies in LMG-treated rats were similar to those of control rats and that the majority of the independent mutations in treated rats were base pair substitutions, with a mutation spectrum similar to that found for control rats. In addition, MG did not induce *cII* mutations in female Big Blue mice. These data suggest that, in the rat, MG might be promoting spontaneous lesions in a manner similar to that reported in the studies on initiation/promotion of hepatic preneoplastic lesions and tumours in male Wistar rats using DEN as an initiator and MG as a promoter.

However, in female mice fed diets containing LMG for 104 weeks, a dose-related trend in increased incidence of hepatocellular adenomas or carcinomas was reported, with the incidence being statistically significant in the highest dose group. Although hepatocellular adenomas and carcinomas in mice often are not considered to originate from a DNA-reactive mechanism when they are the only induced tumour form, the induction of *cII* mutations in liver cells by a high dose of LMG in female transgenic mice indicates that a genotoxic mechanism cannot be ruled out. Importantly, the mutation spectrum of *cII* mutations was different from that of the control mice, with a notable increase in G → T and A → T transversions. A number of studies have shown that mutagenic aromatic amines produce predominantly such transversions in Big Blue rodents.

The Committee noted that LMG produced DNA adduct species, the levels of which increased significantly with dose, in the liver of male F344 and female Big Blue rats, but had no carcinogenic effect in the liver of male and female F344 rats. In contrast, in the liver of female B6C3F1 mice, where LMG induced mutations and tumours, the level of DNA adducts was borderline. This suggests that the DNA adduct species formed after treatment with MG and LMG is of low mutagenic and carcinogenic potential. In addition, the induction of *cII* mutations by LMG in the Big Blue mouse appears to be tissue specific, as tumours developed only in the liver of B6C3F1 mice.

### *Preliminary dietary exposure considerations*

The Committee performed preliminary dietary exposure assessments on the basis of two different sets of residue data. The first set represented results of monitoring and surveillance data, and the concentrations found may have resulted from environmental contamination or from illegal uses. The second set of data was taken from a well conducted study using MG under realistic conditions of use in fish of a size ready for human consumption.

For the first approach, the Committee examined a number of reports on monitoring and surveillance data for MG and LMG. Of 3277 samples selected from these reports, 222 samples were reported positive for MG in the range from 0.2 to about 600 µg/kg fish muscle. For many of the results, it is not defined what "malachite green" means (MG, LMG or the sum of both), because the method was inadequate. However, it is possible that some national authorities have more detailed data.

It was found that only a subset of the above data had been systematically collected. These were monitoring data (spanning from 1995 to 2006) published in the United Kingdom on the occurrence of MG and LMG in fish muscle. If both substances were found in a sample, the Committee calculated the sum. The Committee estimated the mean level in the positive samples to be 30.7 µg/kg fish muscle and the level at the 97.5th percentile to be 138 µg/kg. Assuming the daily consumption of fish to be 300 g/person, the daily exposure to the sum of MG and LMG can be calculated to be 9.2 and 41 µg/person at the mean and 97.5th percentile, respectively. For a 60-kg person, this would be equivalent to 0.15 and 0.69 µg/kg bw per day for the mean and 97.5th percentile, respectively.

In the open literature, well conducted residue studies suitable to predict the concentration–time course of residues of MG in fish are available only for two species, the rainbow trout (*Oncorhynchus mykiss*) and the channel catfish (*Ictalurus punctatus*). Only for trout were sufficient individual animal data available to perform a statistical evaluation. The data and the results of the assessment are described below.

A study investigating the metabolite profiles and residues of MG in trout tissues was conducted in trout kept in tanks under tightly controlled conditions. Concentrations in the exposure tanks were maintained at 2 mg/l by a metering apparatus, using $^{14}C$-labelled MG (radiochemical purity 98%) stock solution at 800 mg/l and delivering 10 ml/min of this solution to the tank.

Seventy-two randomly selected trout, each weighing about 350 g, were divided into three groups of 24 fish. After a 1-h exposure, the fish were removed to a depuration tank containing flowing, uncontaminated water. At specific time intervals during exposure and depuration, two or three trouts were removed randomly from each group, and the concentrations of total radioactive residue in tissue homogenates as well as the ratio of MG and LMG concentrations in an organic extract were determined. These data were used by the Committee to calculate the tissue concentrations of MG and LMG for the intake assessment.

The highest concentrations of residues were found in liver and kidney; however, for the intake estimates, muscle was used. In skin, there were also significant concentrations of residues; however, concentrations were not calculated for muscle and skin in natural proportions, as the concentrations in muscle were higher than those found in skin, and therefore the approach using the muscle data was slightly more conservative.

The kinetic data representing the concentrations of MG and LMG over the time period between the end of the treatment and 505 h were subjected to statistical analysis using one exponential term on the basis of the natural logarithms of the residue contents. Depletion half-lives of 28 h for MG and 197 h for LMG were determined. The kinetic parameters, including the variance of the data, were used to calculate model intakes for every day of 80 years of a human lifespan, assuming daily consumption of 300 g of fish muscle. For this purpose, 29 220 approximately log-normally distributed random numbers were generated for each time point of interest ranging from the predicted value of the regression line minus 4 times the residual variance to the same predicted value plus 4 times the residual variance. These calculations were repeated for a number of assumed slaughter times of the fish, ranging from 1 h (end of treatment) to 500 h. The results were expressed in mg MG (LMG)/kg of human body weight. The minima, maxima and several percentiles, including the median of these estimated daily intakes, were calculated. The median was used for an assessment of chronic intake. The median daily intake of LMG ranged from 7.3 μg/kg bw at 1 h to 0.87 μg/kg bw at 500 h (Table 4).

The Committee considered that the assumption of consumption of 300 g of fish contaminated with MG and LMG every day for a lifetime is a highly conservative assumption. In addition, it was assumed that the concentrations of MG and LMG would not change during cooking of the fish.

Table 4
**Results of an intake assessment for malachite green and leucomalachite green**

| | Intake (µg/kg bw per day) at various theoretical slaughter times of fish (h) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 1.6 | 2.4 | 3.8 | 5.9 | 9.2 | 14.3 | 22.4 | 34.9 | 54.3 | 84.7 | 132.0 | 205.8 | 320.8 | 500.0 |
| **MG** | | | | | | | | | | | | | | | |
| Lowest intake | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Median intake | 1.8 | 1.8 | 1.7 | 1.7 | 1.6 | 1.5 | 1.3 | 1.1 | 0.8 | 0.5 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 |
| P90 | 3.8 | 3.8 | 3.7 | 3.6 | 3.4 | 3.1 | 2.8 | 2.3 | 1.6 | 1.0 | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 |
| P95 | 4.7 | 4.7 | 4.5 | 4.4 | 4.2 | 3.8 | 3.4 | 2.8 | 2.0 | 1.3 | 0.6 | 0.2 | 0.0 | 0.0 | 0.0 |
| P97.5 | 5.6 | 5.6 | 5.4 | 5.2 | 5.0 | 4.6 | 4.0 | 3.3 | 2.4 | 1.5 | 0.7 | 0.2 | 0.0 | 0.0 | 0.0 |
| P99 | 6.9 | 6.9 | 6.6 | 6.5 | 6.1 | 5.7 | 5.0 | 4.1 | 2.9 | 1.8 | 0.9 | 0.3 | 0.0 | 0.0 | 0.0 |
| Highest intake | 15.6 | 16.2 | 21.1 | 15.2 | 14.2 | 14.1 | 11.8 | 9.8 | 10.4 | 3.8 | 2.0 | 0.6 | 0.1 | 0.0 | 0.0 |
| | | | | | | | | | | | | | | | |
| MOE for median intake | 11 128 | 11 225 | 11 540 | 11 940 | 12 609 | 13 664 | 15 426 | 18 921 | 25 713 | 41 351 | 86 780 | 279 316 | 1 715 561 | 29 143 431 | 2 364 965 091 |
| | | | | | | | | | | | | | | | |
| **LMG** | | | | | | | | | | | | | | | |
| Lowest intake | 1.5 | 1.4 | 1.5 | 1.3 | 1.5 | 1.1 | 1.3 | 1.2 | 0.9 | 0.8 | 0.8 | 0.5 | 0.3 | 0.3 | 0.1 |
| Median intake | 7.3 | 7.3 | 7.2 | 7.1 | 6.9 | 6.7 | 6.4 | 6.0 | 5.5 | 4.8 | 4.0 | 3.3 | 2.5 | 1.7 | 0.9 |
| P90 | 12.6 | 12.6 | 12.3 | 12.2 | 12.0 | 11.7 | 11.3 | 10.7 | 10.0 | 8.9 | 7.8 | 6.4 | 4.8 | 3.3 | 1.7 |
| P95 | 14.7 | 14.7 | 14.5 | 14.6 | 14.1 | 13.8 | 13.3 | 12.6 | 11.7 | 10.7 | 9.3 | 7.7 | 5.8 | 4.0 | 2.1 |
| P97.5 | 16.9 | 17.0 | 16.6 | 16.8 | 16.4 | 15.8 | 15.3 | 14.5 | 13.5 | 12.5 | 11.0 | 9.1 | 6.9 | 4.6 | 2.4 |

Table 4 (contd)

| | Intake (µg/kg bw per day) at various theoretical slaughter times of fish (h) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1.0 | 1.6 | 2.4 | 3.8 | 5.9 | 9.2 | 14.3 | 22.4 | 34.9 | 54.3 | 84.7 | 132.0 | 205.8 | 320.8 | 500.0 |
| P99 | 19.7 | 19.5 | 19.6 | 19.7 | 19.1 | 18.9 | 18.3 | 17.3 | 16.0 | 15.0 | 13.4 | 10.9 | 8.3 | 5.6 | 3.0 |
| Highest intake | 48.5 | 42.4 | 36.0 | 39.7 | 36.2 | 34.6 | 38.0 | 38.9 | 31.5 | 32.0 | 30.3 | 22.6 | 16.6 | 14.3 | 6.4 |
| MOE for median intake | 2 750 | 2 746 | 2 792 | 2 832 | 2 902 | 2 976 | 3 108 | 3 337 | 3 653 | 4 159 | 4 958 | 6 071 | 8 076 | 12 095 | 22 891 |
| | | | | | | | | | | | | | | | |
| **Sum** | | | | | | | | | | | | | | | |
| Lowest intake | 1.7 | 1.6 | 1.7 | 1.4 | 1.7 | 1.3 | 1.4 | 1.3 | 1.0 | 0.8 | 0.8 | 0.5 | 0.3 | 0.3 | 0.1 |
| Median intake | 9.1 | 9.1 | 8.9 | 8.7 | 8.5 | 8.2 | 7.7 | 7.1 | 6.3 | 5.3 | 4.3 | 3.4 | 2.5 | 1.7 | 0.9 |
| P90 | 16.4 | 16.4 | 16.0 | 15.8 | 15.4 | 14.8 | 14.1 | 12.9 | 11.6 | 9.9 | 8.3 | 6.6 | 4.9 | 3.3 | 1.7 |
| P95 | 19.4 | 19.4 | 19.0 | 19.0 | 18.3 | 17.6 | 16.7 | 15.4 | 13.8 | 12.0 | 9.9 | 7.9 | 5.9 | 4.0 | 2.1 |
| P97.5 | 22.5 | 22.5 | 22.0 | 22.0 | 21.4 | 20.4 | 19.3 | 17.8 | 15.9 | 14.0 | 11.7 | 9.3 | 7.0 | 4.6 | 2.4 |
| P99 | 26.7 | 26.4 | 26.2 | 26.3 | 25.2 | 24.6 | 23.3 | 21.4 | 18.9 | 16.8 | 14.3 | 11.2 | 8.4 | 5.6 | 3.0 |
| Highest intake | 64.1 | 58.6 | 57.1 | 54.9 | 50.4 | 48.8 | 49.8 | 48.7 | 42.0 | 35.8 | 32.3 | 23.2 | 16.7 | 14.3 | 6.4 |
| | | | | | | | | | | | | | | | |
| MOE for median intake | 2 205 | 2 206 | 2 248 | 2 289 | 2 359 | 2 444 | 2 587 | 2 836 | 3 198 | 3 779 | 4 690 | 5 942 | 8 038 | 12 090 | 22 891 |

P, percentile.

*Evaluation*

The Committee first addressed the question of the use of MG for food-producing animals. There are no conventional studies available on the absorption, distribution, metabolism and excretion of MG in mammalian species. Although the available short- and long-term studies point to a NOAEL in the order of 10 mg/kg bw per day, the study on teratogenicity in rabbits, albeit of low quality, raises concern regarding the potential developmental toxicity of MG. Because a NOAEL could not be identified, additional studies would be needed to properly address the potential reproductive and developmental hazards of MG. In addition, following ingestion, MG is expected to be extensively reduced to LMG, primarily by the gastrointestinal microflora, before absorption, and it cannot be ruled out that LMG, the major metabolite of MG, induces hepatocellular adenomas and carcinomas in female mice via a mutagenic mode of action. Based on these considerations, the Committee considered it inappropriate to establish an ADI for MG. Therefore, the use of MG for food-producing animals cannot be supported.

The Committee thereafter evaluated the safety of residues of MG and LMG in fish as a result of industrial use and other sources of contamination. The Committee considered whether it could establish a margin of exposure (MOE) for non-cancer end-points. In view of the deficient database, the Committee considered it inappropriate to derive an MOE for non-cancer end-points for MG and LMG.

In the evaluation of exposure to genotoxic and carcinogenic residues, the Committee considered the induction of hepatocellular adenomas or carcinomas in female mice treated with LMG to be the pivotal effect for the risk assessment of MG and LMG as contaminants in food. Because there is no information on the conversion rate of MG to LMG in food, the Committee considered it prudent to evaluate the sum of MG and LMG in food expressed as LMG.

For substances that are genotoxic and carcinogenic, JECFA (contaminants) has suggested using an MOE approach in the risk assessment of unintentional contaminants (Annex 1, reference *176*). The present Committee agreed to this approach. The MOE is the ratio between a defined reference point on the dose–response curve for the adverse effect and the human intake of the substance. As a reference point from the dose–response curve for the pivotal adverse effect, it was suggested that the $BMDL_{10}$, which is the lower limit of a one-sided 95% confidence interval on the benchmark dose (BMDL) calculated for a benchmark response of 10% incidence above the modelled background incidence, be used.

The United States Environmental Protection Agency's benchmark dose (BMD) software (BMDS) version 1.4.1 was used for modelling the liver tumour dose–response (hepatocellular adenomas and carcinomas combined) in the LMG-treated female mice. The following dose–response models were fitted to the dose–incidence data: gamma, logistic, log-logistic, multistage, probit, log probit, quantal linear and Weibull models. The BMD and BMDL values for an extra 10% risk compared with the modelled background incidence ($BMD_{10}$ and $BMDL_{10}$) were estimated by performing 250 iterations.

The $BMD_{10}$ values from the accepted models ranged from 33.5 to 43.1 mg LMG/kg bw per day, and the $BMDL_{10}$ values ranged from 18.5 to 31.2 mg LMG/kg bw per day. In order to be prudent, the Committee decided to use the more conservative lower end of this range of values for the evaluation and, to simplify the calculation, chose a $BMDL_{10}$ value of 20 mg LMG/kg bw per day as the reference point for the MOE calculation.

Assuming a daily consumption of 300 g of fish contaminated with MG and LMG, the estimated exposure to the sum of MG and LMG for a 60-kg person was 0.15 μg/kg bw per day, expressed as LMG, for the average intake and 0.69 μg/kg bw per day for the high (97.5th percentile) intake. Comparison of these mean and high-level exposures with the $BMDL_{10}$ of 20 mg/kg bw per day indicates MOEs of about 130 000 and 30 000, respectively. JECFA has previously, at its sixty-fourth meeting (Annex 1, reference *176*), considered MOEs of 10 000 or higher for unintended contaminants (polycyclic aromatic hydrocarbons and ethyl carbamate from food, excluding alcoholic beverages) to be of low concern for human health.

The Committee also performed preliminary estimates of the potential exposures in the case that MG had been used to treat fish in aquaculture and the fish had been subjected to various depletion times. For the median intake, the sum of MG and LMG was about 9 μg/kg bw per day at the end of the 1-h treatment period and about 1 μg/kg bw per day after a depletion time of 500 h. Comparison of these exposure levels with the $BMDL_{10}$ of 20 mg/kg bw per day indicates MOEs of about 2000 and 20 000, respectively. The 97.5th percentile intakes were about 23 and 2 μg/kg bw per day at the end of the 1-h treatment period and after a depletion time of 500 h, respectively, providing MOEs of about 900 and 10 000 (see Table 4).

The current Committee noted the conclusion at the sixty-fourth JECFA and agreed that MOEs of less than 10 000 for genotoxic and carcinogenic contaminants indicate a health concern.

A toxicological monograph was prepared.

### *Residue evaluation*

*Data on pharmacokinetics and metabolism*

Pharmacokinetic studies exhibiting several technical and scientific limitations were performed in rainbow trout. MG was administered through uptake from the water bath. The heavily vascularized gill was assumed to be the principal site of MG uptake from solution.

In one of the few well designed and well conducted studies, the pharmacokinetics and metabolism of MG in channel catfish were studied after intravascular administration of 0.8 mg of $^{14}C$-labelled MG. For waterborne exposures, the initial dye concentration was 0.8 mg/l, and animals were exposed for 1 h. The channel catfish had body weights ranging from 0.5 to 0.7 kg. For the collection of blood and urine, the dorsal aorta and urinary bladder were cannulated. Tissue distribution was determined after waterborne exposure in groups of five animals killed at 2, 4, 24, 96, 168 and 336 h. Additional animals dosed with unlabelled MG were sacrificed after 24 and 42 days. MG and LMG plasma and muscle concentrations were determined, as well as total radioactivity.

After intravascular dosing, MG plasma concentrations declined rapidly; simultaneously, LMG plasma concentrations increased, to reach a peak of 0.88 mg/l 0.75 h after dosing. At this time point, the corresponding concentration of MG was 0.6 mg/l. At 10 h, the concentrations of MG and LMG were 0.05 and 0.2 mg/l, respectively. The sum of these two compounds accounted for approximately 70% of the total drug equivalents at each sampling time.

After waterborne exposure at 21 °C, plasma total radioactivity increased rapidly. The concentrations of MG and LMG reached 2.77 and 1.56 mg/l, respectively, at the end of dosing. While MG plasma concentrations decreased rapidly after transfer of fish to clean water, LMG plasma concentrations increased to a maximum of 2.36 mg/l 1 h after transfer. Ten hours after the end of treatment, the MG concentration in plasma had declined to the LOD of 0.25 mg/l. At this time, the LMG concentration was estimated to be 30 times higher. LMG plasma concentrations were still 0.11 mg/l at day 14 after treatment.

The mean recovery of total radioactivity from muscle of treated fish was 88%. MG and its metabolites were widely distributed and concentrated in tissue, with the highest total residue concentrations in the excretory tissues and fat and the lowest in the muscle and plasma. At the end of the exposure period, MG and LMG concentrations in muscle were 1.18 and 1.45 mg/kg, respectively. Fourteen days after treatment, concentrations were 0.01 and

0.52 mg/kg, respectively. LMG was quantifiable for up to 42 days in muscle. Three unidentified metabolites eluting before LMG were detected on the HPLC system. The sum of the concentrations of these metabolites reached a maximum of 31.3% of the total residue at 24 h after treatment. The effect of pH of the exposure solution was studied at pH values of 6, 7 and 8. Uptakes increased with pH, which may be due to the change in equilibrium concentrations between MG and its carbinol form and in the rates of conversion of MG into carbinol.

No systematic metabolism study was available in fish.

### *Residue data*

*Eggs and fry*. MG accumulates in the eggs of gravid female salmon after treatment and remains detectable in eggs and newly hatched fry. Rainbow trout eggs were treated by repeated flush treatment, at an expected concentration of 1 mg/l of $^{14}$C-labelled MG, every 3 days for 31 days. A flush treatment consisted of adding a concentrated solution of MG to an incubation unit and flushing it through with fresh water. Total radioactivity was determined in five eggs, and the MG concentration was determined in five other eggs by HPLC with post-column oxidation and visible light detection. The pretreatment concentration of radioactivity in eggs and fry increased from day 0 to day 31 to a concentration of 271 ± 42 mg/kg, without reaching a steady state, and declined to 55 ± 11 mg/kg on day 28 after the final treatment. LMG was the major residue. One more polar unknown compound was found in addition to the known compound.

*Fish*. Trout, with an approximate body weight range from 200 to 300 g, were exposed for 24 h to MG, added in a tank at an initial concentration of 0.2 mg/l. The final MG concentration in water was 0.005 mg/l after 24 h. At the end of the treatment, the total concentration of MG plus LMG in fish muscle determined by HPLC was 910 ± 243 µg/kg, and the MG concentration was 86.3 ± 54.4 µg/kg. On subsequent days, the concentrations of the parent drug rapidly decreased according to a biphasic time–concentration curve, and the between-fish variability increased. The depletion of LMG in muscle comes closer to a log-linear curve. The concentrations measured in the fatty tissue were very high, and a very high correlation was found among the fat content of muscle, the concentration of LMG in muscle and the rate of depletion of MG-related residues.

Juvenile tilapias (*Oreochromis niloticus*) (body weight = 24.1 ± 6.8 g) were exposed to MG at two therapeutic doses (0.1 g/l water for 24 h and 0.2 g/l water for 1 h). The fish were then transferred into clean water and collected at different sampling times until 360 h post-exposure. An LC-MS/MS method with an LOD of 2 µg/kg was used to determine MG and LMG

concentrations in muscle tissue. The highest concentrations of MG and LMG were 35.6 ± 5.8 μg/kg and 32.2 ± 17.5 μg/kg, respectively. The MG level decreased to 0.42 ± 0.15 μg/kg within 24 h, whereas LMG was still present after 120 h at a level of 1.48 ± 0.7 μg/kg. After treatment at the lower dose, the highest average concentrations of MG and LMG were 4.6 ± 1.8 μg/kg and 30.6 ± 2.6 μg/kg, respectively. The concentration of the parent MG was 2.0 ± 0.35 μg/kg at 72 h and not detectable 168 h after treatment. Concentrations of LMG remained stable between approximately 12 and 72 h after treatment. At 360 h after exposure, the average concentration was 3.5 ± 2.3 μg/kg.

*Analytical methods*

Two strategies are currently followed for MG and LMG detection in fish tissue. The first is based on the measurement of each molecule separately, and the second is based on measurement of the molecules together after conversion of LMG into MG by chemical oxidation. Because of the instability of MG and LMG, particular precautions are taken throughout the protocol (e.g. amber flasks to prevent photodegradation; antioxidants/reductants and radical scavengers to avoid redox reactions). MG and LMG are generally extracted from fish tissue with an acetonitrile–buffer mixture and are then purified on SPE cartridges. Because of their notable polarities, MG and LMG are analysed by HPLC (reversed-phase), the mobile phase being based on an acetonitrile–buffer mixture. MS is the most widespread detection technology used, because it provides adequate sensitivity and specificity. Ionization is generally performed by atmospheric pressure ionization (either ESI or APCI sources), and positive ions are exploited. The signal acquisition—by selected reaction monitoring—is realized on triple quadrupole or ion trap detector. Isotope dilution approaches ($^{2}H_{5}$-MG and $^{13}C_{6}$-LMG) are used for quantification of MG and LMG, providing accurate quantification by overcoming pitfalls mainly due to matrix suppression during ESI. HPLC coupled to a UV or fluorescence detector has been reported, but it never reached the global performances of MS. Post-column oxidation (e.g. with lead dioxide) of LMG to MG is often reported when UV (conversion of the colourless leuco form into the coloured form) or MS (detection of MG is more sensitive than that of LMG) is used as a detector. Methods are generally validated according to internationally recognized requirements. When LC-MS/MS (+ESI) is used, decision limits (CCα[1]) are in the 0.1–1.0 μg/kg range, and detection capabilities (CCβ[2]) are in the 0.2–2.0 μg/kg range. There is good linearity of the

[1] CCα means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

[2] CCβ means the smallest content of the substance that may detected, identified and/or quantified in a sample with an error probability of β.

response in the range 0.5–10 µg/kg on triple quadrupoles or ion trap detectors, especially when labelled MG and LMG are used as internal standards.

Main issues with the analytical technique concern the recovery of MG/LMG from the sample and their stability throughout the analytical process. The recovery of MG in tissue may be improved by the development of high-pressure solvent extraction techniques and/or enzymatic/chemical digestion of the tissue. Dramatic degradation is reported at room temperature for MG, and MG and LMG recoveries are strongly affected by freeze–thawing cycles. LMG is not the end-point of MG degradation; indeed, *N*-demethylation, hydroxylation and cleavage of the conjugated structure leading to benzophenone derivatives have been described. More than 20 transformation products have been identified. These reactions occur both in living fish organisms during enzymatic action and also during photo-oxidative degradation. Some identified degradation products in incurred rainbow trout are *N*-demethyl-MG, *N*,*N*-didemethyl-MG, *N*-demethyl-LMG and *N*,*N*-didemethyl-LMG. These derivatives were found to represent about 20% of the total concentration of MG residues. Degradation products of MG and LMG might be prevented or at least reduced during sample preparation by addition of ad hoc antioxidant agents to the analytical matrix.

*Maximum residue limits*

MRLs for MG and LMG could not be recommended by the Committee.

A residue monograph on MG and LMG was prepared.

## 3.4 Melengestrol acetate

### *Explanation*

Melengestrol acetate (17α-acetoxy-6-methyl-16-methylene-4,6-pregnadiene-3,20-dione; MGA) is a synthetic progestogen that is active after oral administration. It is administered as a feed additive to female beef cattle (heifers) to improve the efficiency of feed conversion, promote growth and suppress estrus. The range of approved doses is 0.25–0.50 mg/heifer daily, and it is fed for the duration of the fattening/finishing period, usually 90–150 days.

MGA has been reviewed previously by the Committee at its fifty-fourth, fifty-eighth, sixty-second and sixty-sixth meetings (Annex 1, references *146*, *157*, *169* and *181*). An ADI of 0–0.03 µg/kg bw was established at the fifty-fourth meeting of the Committee. MRLs were initially recommended at the fifty-fourth meeting of the Committee and subsequently reconsidered at the sixty-second and sixty-sixth meetings based on new metabolism studies that identified specific metabolites and determined their progestogenic activity

relative to that of MGA. The sixty-sixth meeting recommended MRLs of 18 µg/kg in fat, 10 µg/kg in liver, 2 µg/kg in kidney and 1 µg/kg in muscle tissue of cattle. These MRLs are expressed as MGA equivalents and cover both MGA itself, as the marker residue, and its metabolites. The biological activity of the metabolites (non-MGA fraction of the tissue residues) was converted to MGA equivalents, taking into account their known, lower progestogenic activity and converting the marker residue to total residue, based on the fraction attributable to marker residue in each of the relevant tissues. The analytical methods were extensively reviewed at the fifty-eighth meeting of the Committee, at which time the Committee noted that the available HPLC-MS method submitted by the sponsor was suitable to measure residues in food animal tissues.

At the 17th Session of CCRVDF (*7*), CCRVDF recommended that MGA be reconsidered based on a commitment of new scientific data on toxicity and hormonal effects to be provided by the European Commission, including reference to the specific issues to be addressed. Scientific data have now been submitted by the European Commission, comprising a number of papers published in the scientific literature or submitted as unpublished original research reports to the European Commission between 1996 and 2007. JECFA was asked to reconsider MGA taking into account the more recent data on analytical methods, measurement of residues in tissues, toxicity and hormonal effects of MGA.

In the covering letter included in the European Commission submission, the Committee was asked in particular:

- To reconsider important end-points, such as the effects on pre-pubertal children (and other subpopulations that may be exposed) on the immune system, endocrinological effects etc.
- To discuss data indicating that hormones do not only act via hormone-mediated receptors but other mechanisms (e.g. direct and indirect genotoxic effects).
- To re-calculate the data from residues in meat from animals treated with these hormones for growth promotion purposes and to take into account data coming in particular from improved analytical capacities.
- To describe precisely what authorised use of g*ood practice of use of the veterinary drugs* it considers appropriate in the evaluation and their respective impact on potential exposure and risk estimate.

These issues were considered by the Committee at its present meeting, taking into account relevant earlier information as well as the submitted data. The Committee also searched the literature for any new publications on MGA since its last toxicological review in 2000 (Annex 1, reference *146*). The

majority of the papers and reports submitted by the European Commission concerned actions of hormones or substances with hormone-like activity in general. There is only limited new or additional information on MGA itself, covering developments in analytical methods for residue analysis, further information on plasma concentrations of MGA and MGA residues in tissues of heifers, the mode of action of MGA in accelerating onset of puberty in heifers, lack of effect of MGA in bulls, further in vitro studies on interactions of MGA with hormone receptors and downstream effects, pathological effects in zoo animals with long-term contraceptive MGA implants, and effects of prenatal, juvenile and adult exposure to MGA in rabbits.

The main focus of this re-evaluation is on MGA itself. The Committee did not consider it necessary to review the considerable amount of information that is now available from studies on experimental animals, wildlife and humans concerning the possible effects of a wide range of hormonally active chemicals on development, physiological function and disease (endocrine disrupter effects), as a number of general reviews have addressed these issues. This re-evaluation of MGA is predicated on an understanding that the present state of the science provides strong evidence for effects of endocrine disrupters in laboratory animals. However, while there are serious concerns for wildlife and human health effects, the causal links between exposure and endocrine disruption in these populations remain unclear. As others have pointed out, attempting to relate events during fetal and neonatal life to subsequent function and adult disease is an exceedingly difficult challenge for epidemiology. The Committee also noted that there is still no consensus in the scientific community as to whether low-dose exposures to endocrine disrupters cause adverse effects in humans.

Since MGA has both progestogenic and glucocorticoid activity, the Committee gave specific consideration to background data on the possible role of progesterone and interactions of progestogens with progesterone receptors in development and disease and to the possible influence of glucocorticoids on immune function. The Committee noted that while MGA undoubtedly has been shown to have hormonal activity, as demonstrated by in vitro experiments and in vivo studies in laboratory animals and treated cattle, an important consideration in the risk assessment is whether human exposure to residues in meat at the upper bound of the ADI (0.03 μg/kg bw) would be expected to have any effect.

### *Toxicological evaluation*

The progestogenic activity of MGA in humans was reviewed by the Committee in 2000 (Annex 1, reference *147*). Steroid receptor specificity and relative binding activity of MGA and its four major metabolites were also

reviewed by the Committee in 2004 (Annex 1, reference *169*), when it was concluded that MGA and its metabolites exert their biological action primarily as progestogens and secondarily as glucocorticoids, with no androgenic or estrogenic activity at relevant physiological concentrations. The Committee noted in 2004 that the most potent progestogenic metabolite of MGA, 2β-hydroxy-MGA, had a potency of only 12% compared with that of MGA itself. In the present evaluation, additional in vitro studies on progesterone receptor binding affinity were reviewed, and they are consistent with the previous data. Overall, the studies indicate that the relative progesterone receptor binding affinity of MGA is greater than that of progesterone by around 5- to 11-fold, depending on the assay, whereas the metabolites of MGA have lower affinities than progesterone.

The first area the Committee was asked to reconsider was the important toxicological end-points in relation to particular subpopulations that may be exposed. The Committee considered the hormonal activity profile of MGA in relation to prenatal and postnatal development, genotoxicity, cancer and immunotoxicity. Potential pharmacological and toxicological effects have been considered.

The Committee noted that many of the general concerns that have been expressed concerning the possible effects of endocrine disrupters on fetal and child development relate to estrogenic, androgenic and anti-androgenic activity of substances. MGA is not considered to show estrogenic activity in vivo and has estrogenic activity only at high concentrations (nanomoles to micromoles per litre) in vitro. In this regard, the Committee noted that many of the data submitted by the European Commission referred to the known or possible effects of estrogens, which are not considered relevant for MGA. Nor is MGA considered to have anti-estrogenic activity, from its lack of activity in the human breast cancer cell line, MCF-7 cells, or (anti)-androgenic activity, from its lack of binding to the androgen receptor.

Concerning progestogenic activity, one way of assessing whether exposure to residues of MGA and its metabolites in meat would have any effect in adult humans is to compare the pharmacological activity of progestogenic steroids that are structurally related to MGA, as was also done earlier by the Committee (Annex 1, reference *147*). MGA has not been used in human therapeutic medicine, but the closely related compounds megestrol acetate (MA) and medroxyprogesterone acetate (MPA) are, or have been, used in human contraception and for the treatment of endometriosis and cancer (breast, endometrium, ovary, testis). The amounts of MA or MPA used orally in endometriosis and cancer therapy are in the range of 30–800 mg daily. The amounts of MPA used orally for contraception are lower, in the range of 2.5–10 mg/day, whereas contraceptive doses of MA have been reported to be

in the range of 0.35–0.5 mg/day. MGA has been estimated to be less potent than MA, based both on inhibition of menstruation in estrogen-primed women and on alteration of cervical mucus. The relative binding affinity of MPA to the bovine progesterone receptor, compared with that of progesterone, is 223%, whereas that of MGA is 526%. Data from humans and laboratory animals indicate that MGA is about 4 times more potent than MPA with respect to activity on the endometrium. This information, together with that on the lowest pharmacologically active doses of MPA in the range of 2.5–10 mg daily, suggests that amounts of orally ingested MGA in humans would need to be of the order of 0.5 mg/day (8 μg/kg bw for a 60-kg person) or more to exert any discernible pharmacological effect. This is supported by limited data showing that 7.5 or 10 mg MGA/day, but not 5 mg/day, delays the onset of menstruation in regularly ovulating women, and that 2.5 mg MGA/day induces withdrawal bleeding in estrogen-primed amenorrhoeic women. Ingestion of MGA at the level of the upper bound of the ADI (1.8 μg/day for a 60-kg person) would be around 300-fold below 0.5 mg/day, the estimate of the minimal or no-effect level for alteration of menstrual cycle hormones and endometrial effects of MGA in women. The ADI for MGA is derived by application of an uncertainty factor to the minimally effective progestogenic dose of 5 μg MGA/kg bw per day affecting the menstrual cycle in cynomolgus monkeys, which appear to be the most sensitive species.

Data on the role of progesterone during embryonic and fetal development and on development of the reproductive tract in particular are sparse in comparison with the information that is available on estrogenic, androgenic and anti-androgenic substances. Although there are laboratory animal studies and occasional human case-reports of adverse effects of prenatal or perinatal exposure to high doses of progesterone and other progestogens, data from human trials on the use of progestogens in pregnancy (e.g. to prevent spontaneous abortions) do not provide any evidence of adverse effects on the embryo, fetus or young child.

Data on the effects of MGA on the immune system are limited, and no new information was submitted. The information reviewed by the Committee in 2000 (Annex 1, reference *147*) indicated that there are measurable effects on adrenal gland size, cortisol secretion and susceptibility to infection in cattle given 0.5 mg MGA/day in the feed. Data from clinical trials in humans given MGA indicated that 10 mg/person (167 μg/kg bw) was without any effect on adrenal hormonal responsiveness, a NOEL that is at least 10 000 times higher than the exposure would be at the upper bound of the ADI (0.03 μg/kg bw). These exposure and dose considerations indicate that any indirect effect of MGA through glucocorticoid alteration of the immune system is unlikely. The Committee also noted that in the mammalian repeat-dose studies considered in its previous evaluation, immune system parameters, such as

serum cortisol, leukocyte counts, and adrenal, thymus and spleen weights, were affected by MGA only at doses of 50 μg/kg bw per day or more. Similarly, in three special studies on immunotoxicity in the rat, also considered in the previous evaluation, MGA showed immunosuppressive activity only at doses of 5 mg/kg bw per day or more. Overall, the data show that the effects of MGA on immune system parameters are apparent only at doses that are 6–600 times higher than the dose at which MGA is considered to have minimal progestogenic activity in humans (8 μg/kg bw).

The second area the Committee was asked to consider concerned the data indicating that hormones may act not only by interaction with hormone receptors and the consequent downstream effects, but also by other mechanisms, such as direct or indirect genotoxic activity. This is an important question, because it raises the possibility that adverse effects such as cancer might be incurred through toxic modes of action that are without a discernible threshold.

The Committee noted that in 2000 it had reviewed a comprehensive set of genotoxicity tests on MGA itself, including in vitro tests on gene mutation in bacteria and mammalian cells, unscheduled DNA synthesis in rat hepatocytes and single strand breaks in mammalian cells and an in vivo test for micronucleus formation in mouse bone marrow. The results were all negative, and the Committee had concluded that MGA was not genotoxic (Annex 1, reference *147*). New data on genotoxicity submitted for the present evaluation have shown that MGA does not induce gene mutations or micronuclei in mammalian cells in vitro, nor does it induce *lacI* mutations in *E. coli*. A review published in 2004 on the genotoxicity of hormonal steroids showed that the majority of progestogens are not genotoxic. However, some progestogens that are structurally related to MGA, sharing the 17-hydroxy-3-oxo-pregna-4,6-diene structure, do have genotoxic potential. Since the genotoxic profile of MGA itself is uniformly negative, it appears to differ from some of those with which it shares a structural similarity. The Committee reaffirmed its previous view that the data on MGA show that it is not genotoxic. Thus, there is no evidence that MGA would increase the risk of cancer by a genotoxic mechanism.

However, progesterone, after binding to progesterone receptors and triggering downstream gene expression, clearly plays a role in, for example, human breast development and progression of some types of breast cancer. The Committee also recalled that several long-term studies in mice, reviewed in its previous evaluation, showed dose-related increases in the incidence of mammary adenocarcinoma, but that mechanistic studies had shown that the effect was attributable to promotion caused by stimulation of prolactin secretion, with a NOEL for mammary tumorigenesis of 0.5 mg/kg bw per day.

Epidemiological evidence both from combined estrogen–progestogen therapy and from combined estrogen–progestogen hormone replacement therapy shows that the addition of the progestogen confers a small, but significant, increase in the risk of breast cancer, which is greater than that in users of estrogen alone. Since duration of exposure is a significant risk factor for breast cancer in women taking combined oral contraceptives or combined hormone replacement therapy and the increase in risk for breast cancer is largely confined to current or recent users, this also suggests that the progestogenic component is acting more as a promoter than as an initiator of cancer, which is consistent with a non-genotoxic mode of action. Although the increase in risk of breast cancer is seen in association with pharmacologically active doses of progestogen, the doses used are such that the data do not allow no-effect levels for the increase in risk to be established. However, exposure to MGA from residues in meat is several orders of magnitude lower than the pharmacologically active doses of a progestogen that would elevate the risk of breast cancer. Given the involvement of progesterone receptors in the carcinogenic effects of progestogens, there should be no increase in the risk of cancer at exposure levels below those that activate progesterone receptors in vivo in humans. An additional consideration is that absorption of low concentrations of MGA present as residues in meat would be followed by extensive and rapid metabolism in the liver and that the metabolites of MGA have less binding affinity for progesterone receptors than progesterone itself. The Committee also noted that very young infants, in whom metabolizing capacity is not fully developed, would not be consuming meat or other animal tissues in which MGA residues may be present.

It can be concluded that progestogens do not have a carcinogenic effect on the uterus, since all the available clinical and epidemiological data show that progestogens oppose the hyperplastic effect of estrogens in the endometrium and reduce the likelihood of endometrial cancer in women also exposed to exogenous estrogens. Use of combined estrogen–progestogen oral contraceptives reduces the risk of ovarian cancer and increases the risk of cervical cancer, whereas the data from use of combined estrogen–progestogen hormone replacement therapy are inadequate for evaluation with respect to these two cancer sites. The influence of the progestogen component with respect to these two cancer sites has not been separately analysed.

The fourth area the Committee was asked to consider was the authorized uses according to good practice in the use of veterinary drugs that it considers appropriate in the evaluation and their respective impact on potential exposure and risk estimates. Good practice in the use of veterinary drugs is the official recommended or authorized usage, including withdrawal periods, approved by national authorities of veterinary drugs under practical conditions (*3*). In line with established practice in JECFA, when assessing exposure

to residues and risk, the Committee considers only maximum residues from approved treatments. In the case of MGA, the Committee considered only the approved use in heifers at the recommended dose range of 0.25–0.5 mg/heifer per day for recommending MRLs. The Committee did note that in the case of MGA, which is added to the feed, if the level of addition increases to 3 times the maximum recommended dose of 0.5 mg/heifer per day, then the hormonal activity profile changes, and the desired effect (growth promotion) does not occur.

### *Evaluation*

In reaching its conclusions, the Committee considered not only the data on MGA from its previous toxicological evaluation in 2000 and the few new studies on MGA that have been published or submitted since then, but also more general information on the role of progestogens in human reproduction, prenatal and child development, and cancer. The issue of immunotoxicity was also revisited. The Committee noted previously that MGA has both progestogenic and glucocorticoid activity, and that both earlier and new data confirm these as the principal hormonal activities of MGA, with only weak estrogenic activity shown at relatively high concentrations in vitro.

There are no data on plasma concentrations of MGA following human dietary exposure. However, based on the comparative data considered in the Committee's previous evaluations (Annex 1, references *146* and *169*) showing similarities in absorption and metabolism of MGA among cattle, rats, rabbits and humans, it can be estimated that the plasma concentration in humans after ingestion of 0.03 μg/kg bw (equivalent to the upper bound of the ADI) would be around 0.5–1 pg/ml. This is some 4000-fold below the minimum concentration necessary to stimulate proliferation in the human breast cancer cell line, MCF-7 cells, which is a sensitive indicator of estrogenic activity. In addition, a study in rabbits found concentrations of MGA in plasma in the low nanogram per millilitre range following an oral dose of 0.5 mg/kg bw. This is in the same range as the minimum concentration of MGA necessary to produce effects on the estrogen receptor in MCF-7 cells. Given that the dose in this study was some 17 000-fold greater than the maximum intake of humans exposed to residues in meat, assuming consumption at the upper bound of the ADI, even allowing for the uncertainty introduced by species differences (rabbit versus human), it is highly unlikely that dietary residues of MGA will have any estrogenic effects in humans consuming meat from animals treated with this drug. The Committee also noted that MGA is devoid of genotoxic activity both in vitro and in vivo; thus, non-thresholded mechanisms of carcinogenicity are unlikely to play any role.

Concerning progestogenic activity, there is a small, but significant, increase in risk of breast cancer in humans exposed to progestogens in the form of combined estrogen–progestogen oral contraceptives or hormone replacement therapy, and the evidence suggests that the progestogenic component is acting more as a promoter than as an initiator of cancer. On the basis of comparative estimates of progestogenic activity, the exposure to MGA and its metabolites from residues in meat consumed at the upper bound of the ADI is 200–300 times lower than these pharmacologically active doses and below a dose that would produce any measurable effect on progesterone receptors. Although MGA causes mammary tumorigenesis in the mouse, this is attributable to stimulation of prolactin secretion having a promoter activity, and there is a clear NOEL of 0.5 mg/kg bw per day for this effect. This NOEL is more than 15 000 times higher than the exposure would be at the upper bound of the ADI. The Committee therefore concluded that residues of MGA and its metabolites would be unlikely to have any influence on the development of breast cancer.

Concerning glucocorticoid activity and effects on the immune system, there is no new information. However, the Committee noted that the NOEL for adrenal hormonal responsiveness to MGA in humans is at least 10 000 times higher than the exposure would be at the upper bound of the ADI. Similarly, the NOEL for immunosuppressive activity is at least 1000 times higher than the exposure would be at the upper bound of the ADI.

The Committee concluded overall that the new data do not provide any basis to reconsider the ADI. Human exposure to residues of MGA and its metabolites in meat, resulting from the use of MGA as a feed additive in cattle at a daily dose of 0.25–0.5 mg/heifer, would be unlikely to have any adverse effects on adults, children, the embryo or the fetus.

A toxicological monograph was prepared.

### *Residue evaluation*

The Committee was asked to recalculate the data from residues in meat from animals treated with hormones for growth promotion purposes and to take into account data in particular from improved analytical methods. As MGA was the only hormone for growth promotion purposes on the agenda, estimates of exposure are provided only for MGA.

#### *Analytical methods*

In the submission of new scientific data, references to two analytical methods were included (*9*, *10*). The analytical method reported by Daxenberger et al. (*9*) consists of two procedures—one for liver, kidney and muscle using

HPLC-MS and one for fat using GC-MS. The analytical method reported by Hageleit et al. (*10*) is an enzyme immunoassay (EIA) screening method. In both publications, the authors indicated that the methods had been validated based on the European Commission criteria (*11*). The fifty-eighth meeting of the Committee (Annex 1, reference *157*) reviewed these methods in its report as well as an analytical method provided by the sponsor. A review of the open literature did not reveal any additional validated methods for MGA suitable for regulatory use at the recommended MRLs. A comparison of the performance of the three analytical methods is provided in Table 5.

Table 5
**Comparison of method performance for residues of MGA in bovine tissues**

| Authors, year (reference) | Assay system | Tissues | LOD (µg/kg) | LOQ (µg/kg) |
|---|---|---|---|---|
| Merritt & Hamrick, 2001 (*12*) | HPLC-MS | Fat | 0.42 | 1.0 |
| | | Liver | 0.38 | 0.89 |
| Daxenberger et al., 1999 (*9*) | HPLC-MS | Kidney | ND | 1.0 |
| | | Liver | | 1.0 |
| | | Muscle | | 0.5 |
| | GC-MS | Fat | | 5.0 |
| Hageleit et al., 2001 (*10*) | EIA | Fat | 0.4 | 2.0 |
| | | Muscle | 0.05 | 0.1 |

ND, not determined.

*Evaluation*

The Committee concluded that the HPLC-MS and GC-MS methods are suitable for determination of MGA residues in food animal tissues and that the EIA method may be used as a screening method for detection of MGA residues in food animal tissues. The sponsor's method (*12*) demonstrated the same LOD and LOQ as the two methods submitted for the Committee's review.

No residue data were provided in the Daxenburger et al. (*9*) or the Hageleit et al. (*10*) publication. An estimate of residue exposure for MGA was made using United States Department of Agriculture (USDA) publicly available residue data for 2004–2006 and an analytical method with an LOQ of 10 µg/kg (*13*) in fat tissue. A total of 986 samples of fat tissue from heifers collected over a continuous 36-month time period using a statistically designed sampling programme were analysed. Heifers account for, on average, approximately 7% of total meat and poultry production in the USA. Most animals are raised as feedlot animals and are expected to be rather uniform

in production treatments. Results indicated that no samples had residues at or above the recommended MRL of 18 μg/kg for fat tissue. Sixty samples had quantifiable values, with a median residue concentration of 11 μg/kg and a highest residue concentration of 17 μg/kg. The remaining 926 samples were reported as below the LOQ. Assuming exposure using the median value of 11 μg/kg for the 60 quantifiable residues and applying the food basket value of 50 g for fat, exposure to MGA residues would be 0.55 μg/day or approximately 30% of the upper bound of the ADI. Using the median exposure to MGA residues for all samples, again applying the food basket value of 50 g for fat, the median residue level would be approximately 5 μg/kg, assuming that all residues are one half the LOQ (10 μg/kg) of the USDA method. This would result in an exposure of 0.25 μg/day, equivalent to approximately 14% of the upper bound of the ADI.

An addendum to the residue monograph was not prepared.

## 3.5 Monensin

### *Explanation*

Monensin is a polyether carboxylic ionophore antibiotic produced by *Streptomyces cinnamonensis*. The sodium salt is a mixture of four analogues, A, B, C and D, which are produced during fermentation, with monensin A being the major component (98%). Depending on the method of purification, monensin can exist in mycelial, crystalline and recrystallized forms. It is used for the treatment of coccidiosis in poultry (chickens, turkeys and quail) and ruminants (cattle, sheep and goats). Monensin is also used to control ketosis and bloat in cattle and as a growth promoter feed additive in cattle and sheep. Monensin is mainly effective against Gram-positive bacteria. Monensin is not used in human medicine and was therefore not classified as a critically important antibiotic for humans by the 2007 WHO expert meeting on categorization of critically important antimicrobials for human medicine for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use (*14*). Monensin acts by interfering with ion flux across bacterial membranes, causing reallocation of bacterial energy resources to maintaining cellular pH and ion balance rather than growth and reproduction. Effects on feed conversion efficiency may arise from the ability of monensin to shift rumen fermentation towards the more energetically efficient propionate pathway, reduce methane production and increase nitrogen retention by reducing dietary protein deamination and urinary ammonia excretion.

Monensin has not previously been evaluated by the Committee. CCRVDF decided at its 17th Session (*7*) to put monensin on the priority list for evaluation by JECFA.

The present Committee considered data on the pharmacokinetics (including metabolism), acute toxicity, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity, immunotoxicity, cardiovascular and respiratory toxicity, epidemiological findings and microbiological effects of monensin. Additionally, residue depletion studies and analytical methods were reviewed. Many of the studies were conducted prior to the introduction of GLP.

### *Toxicological and microbiological evaluation*

#### *Biochemical data*

The absorption and excretion of monensin have been studied in a variety of species, including rats and dogs. The recovery of monensin in the bile was independent of dose (33–49% in male rats receiving 5 or 40 mg monensin/kg bw and 31–53% in female rats receiving 2 or 16 mg monensin/kg bw).

Monensin rapidly disappears from the serum in rats and dogs, and the highest concentration of monensin is attained in the liver.

In rats and dogs, less than 10% of excreted monensin is the parent compound. Monensin is extensively metabolized in the liver in the majority of animals. In human microsomes, monensin sodium is extensively metabolized (93–99% by 60 min), and its turnover is similar to that in dogs.

Monensin metabolism occurs primarily through *O*-demethylation or hydroxylation of the ionophore backbone and is believed to occur at least in part by CYP3A. Tested monensin metabolites have decreased antibacterial, anticoccidial, cytotoxic, cardiac and ionophoric activity.

The primary excretion route for monensin is the faeces. In rats, a majority of the administered dose (up to 10 mg/kg bw in males, up to 4 mg/kg bw in females) was excreted within 72 h (70–91%), and excretion was independent of dose. Higher doses in rats of both sexes led to decreased excretion of monensin.

#### *Toxicological data*

Crystalline monensin and several preparations of mycelial monensin were tested for toxicity. The Committee evaluated the toxicity of monensin on the basis of the specific form utilized in the studies.

The acute oral toxicity of monensin was moderate to high. The $LD_{50}$ values in mice, rats and rabbits ranged from 22 to 96 mg/kg bw. Signs of toxicity included hypoactivity, ataxia, dyspnoea, ptosis, loss of righting reflex and

muscle weakness. Pairs of monkeys given a single dose of monensin in a range from 20 to 160 mg/kg bw by gavage developed diarrhoea within 24 h at all doses and vomiting and appetite suppression starting at 110 mg/kg bw, but no mortality occurred. The $LD_{50}$ value for mycelial monensin in monkeys is greater than 160 mg/kg bw.

In a GLP-compliant study, male and female mice fed diets containing 0, 37.5, 75, 150 or 300 mg mycelial monensin sodium/kg for 3 months (equivalent to 0, 5.6, 11.2, 22.5 or 45 mg/kg bw per day) showed a dose-dependent decrease in body weight gain at the end of the study, ranging from 27% and 21% in the lowest dose group in females and males, respectively, to 99% in the highest dose group in both sexes. Mean body weights also declined, with decreases ranging from 5% and 8% in the lowest dose group to 29% and 35% in the highest dose group in females and males, respectively. Except for the declines in body weights and body weight gains in the lowest-dose males, all changes were statistically significant. Elevated creatine phosphokinase values were observed for the males in the two highest dose groups and for the females receiving the highest dose. Mild diffuse vacuolation of cardiac myofibres occurred in the highest dose group. Since weight gain was affected in all dose groups, a NOAEL could not be identified from this study.

Several GLP-compliant and non-GLP-compliant oral subchronic toxicity studies were conducted in rats using mycelial or crystalline monensin. In a non-GLP-compliant study, rats were fed diets containing mycelial monensin sodium for 3 months at nominal concentrations of 0, 25, 50, 80 or 125 mg/kg (based on feed analysis, equal to 0, 0.89–2.45, 1.83–4.63, 3.02–7.71 and 4.54–12.05 mg/kg bw per day in males and 0, 1.30–2.55, 2.75–5.83, 4.04–12.83 and 10.17–20.21 mg/kg bw per day in females). Transient dose-dependent decreases in body weight gain, mean body weight and feed consumption were observed in females receiving 50, 80 or 125 mg/kg in the diet. Males receiving 125 mg/kg in the diet also had a transient decrease in body weight gain. The NOAEL for this study was the nominal concentration of 25 mg/kg in the diet. An exact dose could not be determined owing to the wide range of measured concentrations of monensin in the feed.

In four parallel subchronic toxicity studies conducted according to GLP, rats were fed diets containing crystalline monensin sodium or three different preparations of mycelial monensin sodium at 0, 50, 200 or 400 mg/kg (equivalent to 0, 2.5, 10 and 20 mg/kg bw per day) for 3 months. Mortality occurred in four females and one male exposed to the highest dose of mycelial monensin and in one female in the middle dose group. The cause of death could not be determined; however, a relationship with the treatment could not be ruled out. Decreased body weight gain was observed for all forms of monensin starting at 200 mg/kg in the diet and in female rats exposed to the

mycelial forms of monensin at 50 mg/kg in the diet. Doses of 200 and 400 mg mycelial monensin/kg in the diet reduced feed consumption compared with crystalline monensin and led to decreased body weight gain in male rats. Slight quantitative differences in body weight gain and feed consumption between the crystalline and mycelial forms were not considered biologically significant. Focal degeneration and interstitial myositis of the diaphragm and skeletal muscle occurred in higher incidence in treated female rats than in controls; however, overall incidence and severity were low. Owing to the decreased body weight gain at the lowest dose, a NOAEL could not be determined.

In a non-GLP-compliant study, rats of both sexes were exposed in feed to crystalline or mycelial monensin sodium at 0, 50, 200 or 400 mg/kg (equivalent to 0, 2.5, 10 and 20 mg/kg bw per day) for 3 months. The toxicological responses to both forms of monensin did not differ. Three high-dose females (one in the crystalline group, two in the mycelial group) died during the study. A severe reduction in body weight gain was observed for both formulations starting at 200 mg/kg in the diet. A slight, transient decrease in body weight gain was observed for females in the 50 mg/kg diet group for the first 2 weeks of the study. Increased total bilirubin and alkaline phosphatase levels and decreased mean serum glucose and creatinine levels were seen in all animals receiving either form at 400 mg/kg in the diet and in females receiving 200 mg/kg in the diet. Female rats in all treatment groups also had decreased ALT levels. Initial histopathological examination revealed a non-dose-dependent incidence of scattered foci of a few myocardial fibres, with degeneration, necrosis and infiltration of mononuclear cells, particularly in males, in all three dose groups for both forms of monensin. A second independent pathology evaluation concluded that the scattered myocardial lesions were not adverse and that the incidence was similar to the control incidence. The Committee concluded that a NOAEL could not be identified because of a slight, transient reduction in body weight gain in females in the lowest dose group, which became severe and non-transient in the next dose group.

In a non-GLP-compliant study, two dogs per sex per group were administered monensin sodium orally in capsules at daily doses of 0, 2.5, 5, 11 or 25 mg/kg bw for 90 days. Deaths attributed to treatment with monensin occurred in two males of the highest dose group and one female of the second highest dose group. Females of the highest dose group developed ataxia, tremors, loss of muscular control and slight relaxation of the nictitating membrane; therefore, treatment was stopped after 5 days. There were no signs of toxicity in surviving male and female dogs receiving 11 and 5 mg/kg bw per day or less, respectively. Haematology, clinical chemistry, urinalysis, organ weights and gross pathology of all animals were normal, with the exception of transiently elevated serum ALT levels in the second highest dose group. The NOAEL was 5 mg/kg bw per day.

In a second non-GLP-compliant study, dogs were orally administered monensin sodium at doses of 0, 5, 15 or 50 mg/kg bw per day for 91 days in gel capsules. Two males in the highest dose group and one male in the middle dose group did not survive the study and exhibited myopathy of the heart with degeneration of the muscle fibre, macrophage infiltration and visceral congestion. Dogs dosed with 15 and 50 mg monensin sodium/kg bw per day vomited more frequently, lost body weight, had transient increases in lactate dehydrogenase and aspartate aminotransferase (AST) and developed muscular weakness, ataxia, arrhythmias, convulsions and mydriasis. Degenerative changes in striated muscle were observed starting at 15 mg/kg bw per day. A slight loss of body weight was observed in dogs in all dose groups. Since toxic effects were seen at the lowest dose, a NOAEL could not be identified.

In a 1-year study, mycelial monensin sodium was orally administered as an equally divided dose in gel capsules twice daily to dogs at doses of 0, 1.25, 2.5, 5 or 7.5 mg/kg bw. No data on feed intake were reported. Dogs receiving 5 and 7.5 mg/kg bw per day showed transient signs of toxicity, which included hypoactivity, muscle weakness (particularly legs and neck), stilted gait, difficulty standing and anorexia. Increased ALT and creatine phosphokinase levels were observed during the first 2 weeks of monensin administration in dogs receiving 5 and 7.5 mg/kg bw per day, and several dogs in these groups also had periodic increases in ALT and creatine phosphokinase levels throughout the entire study period. A decrease in body weight gain was seen in male dogs receiving 2.5, 5 or 7.5 mg/kg bw per day, which exceeded 10% for the highest dose. No histopathological changes were seen that were related to monensin exposure. Since body weight gain was decreased at the next higher dose, the NOAEL was 1.25 mg/kg bw per day.

Long-term (1- and 2-year) studies were carried out in mice and rats orally administered mycelial or crystalline monensin sodium. All long-term toxicity studies were conducted according to GLP guidelines.

Mice were given mycelial monensin sodium in the diet at concentrations of 0, 10, 25, 75 or 150 mg/kg (equal to 0, 1.2, 3.1, 10.2 and 22.6 mg/kg bw per day for males and 0, 1.4, 3.5, 11.7 and 25.6 mg/kg bw per day for females) for 2 years. Significant decreases in body weight and body weight gain occurred in mice receiving 25 mg/kg in the diet or greater, and a statistically significant, dose-dependent decrease in leukocyte counts was observed in males receiving monensin at concentrations of 25, 75 or 150 mg/kg in the diet. Minimal increases in urea nitrogen, creatinine, bilirubin, AST and creatine phosphokinase occurred with the highest dose. No substance-related deaths, physical signs or behavioural changes were reported. There was no evidence of carcinogenicity that could be attributed to monensin. A NOAEL of 10 mg/kg in the diet (equal to 1.2 mg/kg bw per day) was identified.

Rats were given crystalline monensin sodium in the diet at 0, 25, 56 or 125 mg/kg (equal to 0, 1.14, 2.57 and 5.91 mg/kg bw per day in males and 0, 1.46, 3.43 and 8.68 mg/kg bw per day in females) for 2 years. Body weight and body weight gain were significantly decreased in animals receiving 125 mg/kg in the diet, and a decrease in these parameters was observed during the first 4 months in rats receiving 56 mg/kg in the diet. Feed conversion efficiency was decreased in the animals receiving 56 or 125 mg/kg in the diet, and mean feed consumption was decreased in animals in the highest dose group during the first 5 weeks of the trial. Crystalline monensin sodium did not produce carcinogenicity. The NOAEL was 25 mg/kg in the diet (equal to 1.14 mg/kg bw per day).

In a second study, rats that were exposed to monensin in utero were further exposed to 0, 33, 50 or 80 mg mycelial monensin sodium/kg in the diet for 2 years (equal to 0, 1.40, 2.18 and 3.60 mg/kg bw per day in males and 0, 1.72, 2.86 and 5.02 mg/kg bw per day in females). Survival in both sexes increased in a dose-dependent manner. A transient decrease in body weight was observed at the beginning of the study in all animals receiving 80 mg/kg in the diet and in females receiving 50 mg/kg in the diet. Body weight gain was also significantly decreased during the 1st week in males receiving monensin at 33 and 80 mg/kg in the diet and during the first 2 weeks in females receiving the highest dose. There was a statistically significant increase in feed intake in females in the highest dose group. In utero exposure followed by 2 years of exposure to mycelial monensin sodium at doses up to 80 mg/kg in the diet did not lead to carcinogenicity. As the observed decrease in body weight gain was transient and restricted to the first few weeks of the 2-year study, the effect was not considered to be adverse. The NOAEL was 80 mg/kg in the diet (equal to 3.60 mg/kg bw per day).

Monensin produced negative results in an adequate range of in vitro and in vivo genotoxicity studies. The Committee concluded that monensin had no genotoxic potential.

In a GLP-compliant multigeneration study, mycelial monensin was administered to three generations of rats and their offspring at doses of 0, 33, 50 or 80 mg/kg in the diet (equivalent to 0, 1.6, 2.5 and 4 mg/kg bw per day). Reduced body weight gain was seen in animals of both sexes in every generation and at all doses. There were no significant differences in fertility, litter size, gestation length, parent and progeny survival or sex distribution, and no evidence of teratogenicity was observed. A NOAEL for parental and offspring toxicity could not be determined owing to the reduction in body weight gain in both sexes in every generation and at all doses. The NOAEL for reproductive toxicity was 80 mg/kg in the diet (equivalent to 4 mg/kg bw per day), the highest dose tested.

In a one-generation, non-GLP-compliant reproduction study, female rats received diets containing a non-specified preparation of monensin at concentrations of 0, 100 or 300 mg/kg (equivalent to 0, 5 or 15 mg/kg bw per day) until premating weights achieved 185 g and during pregnancy and lactation. Female body weight was significantly decreased in the highest dose group after 8 days of treatment and remained lower throughout the study. No significant differences in female fertility were observed. All females became pregnant, with the exception of two in the high dose group, which did not show vaginal opening and could not be mated. Weight gain during pregnancy was not affected by treatment. Gestation length, litter size and number of stillborn pups were also unaltered by monensin treatment. The body weights of male and female pups in the highest dose group were reduced from postnatal day 10 until postnatal day 21. Male offspring in the low dose group showed body weight reduction only on postnatal day 21. No external signs of malformation were detected in the pups. Females perinatally exposed to 100 mg monensin/kg in the diet showed a delay of incisor eruption; this effect was not seen in the high dose group. No other treatment-related effects were observed. Owing to effects on body weight in males in the low dose group on postnatal day 21, a NOAEL for developmental toxicity could not be identified.

In a non-GLP-compliant teratogenicity study, pregnant rabbits received monensin sodium at doses of 0, 0.076, 0.38 or 0.76 mg/kg bw per day from gestation days 6 to 18. Decreased maternal feed consumption was observed in the high dose group during treatment only. Doses up to 0.76 mg/kg bw per day did not affect litter size, corpora lutea number, implantation occurrence, fetal viability or resorption occurrence. In addition, sex distribution, progeny survival and mean fetal weights did not differ between groups. Fetal abnormalities occurred in low incidence and were unrelated to monensin treatment. The NOAEL for maternal toxicity was 0.76 mg/kg bw per day, the highest dose tested. The NOAEL for teratogenicity was 0.76 mg/kg bw per day, the highest dose tested.

In dogs that received monensin sodium by gavage at a single dose of 0, 0.138, 0.345, 0.690 or 1.38 mg/kg, coronary artery flow was significantly increased at 0.690 and 1.38 mg/kg bw, whereas heart rate and blood pressure remained unchanged. The elevation in coronary blood flow was maximal 13–17 min after dosing and returned to normal by 30 min. The threshold for pharmacological effects on the heart was 0.345 mg/kg bw. The observed transient increase in coronary blood flow in dogs given a single oral dose of monensin was considered treatment related but not adverse, owing to the absence of effects on blood pressure or heart rate.

Monensin is not used in human medicine. An evaluation of the medical records of employees involved in the manufacture of monensin from 1968 to 2001 provided no evidence of chronic diseases that could be related to monensin exposure. Several employees developed immunoglobulin E (IgE)-mediated allergic responses, including transient urticaria, swelling of the face or tongue, pruritis, chest congestion and chest tightness, which resolved upon their removal from the monensin manufacturing area.

Two case-reports are available in the literature regarding accidental exposure of humans to monensin. In the first case, a 17-year-old boy ingested an unknown amount of monensin sodium; in the second, a 16-year-old boy consumed approximately 500 mg of monensin. In both cases, a similar pattern of toxicity was observed as has been seen previously during overdosing in domestic animal species. Monensin caused rhabdomyolysis, which led to acute kidney failure in both patients and heart failure in the 16-year-old boy. Death occurred in both patients within 11 days of consumption. The primary targets of monensin overdose in humans appear to be skeletal and heart muscles.

*Microbiological data*

A JECFA decision tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with VICH GL36 (*8*) was used by the Committee to determine the impact of monensin residues on the intestinal microbiota.

The Committee evaluated MIC susceptibility, faecal binding interaction and biological activity of monensin metabolites in the assessment of monensin.

Monensin is microbiologically active against some bacterial genera and species representative of the human intestinal flora.

In tests using the higher bacterial inoculum density of $10^9$ colony-forming units (cfu)/ml, monensin exerted little or no antibacterial activity ($MIC_{50}$ values >128 μg/ml) against *Bacteroides fragilis*, other *Bacteroides* species and *E. coli*. This is consistent with the known spectrum of activity for this compound, which has low activity against Gram-negative bacteria. Monensin also exerted very weak activity against *Bifidobacterium* species. Monensin activity was clearly demonstrable against the other six bacterial groups tested at the higher inoculum density. *Peptostreptococcus* was the most susceptible group ($MIC_{50}$ of 0.5 μg/ml), whereas *Fusobacterium* was the least susceptible.

To determine the effect of faecal binding on the antibacterial activity of monensin, selected monensin concentrations of 0, 1, 2, 5, 10, 20, 50 and 100 μg/ml were incubated with increasing concentrations of sterilized human

faeces (0, 10, 20 and 50% weight by volume [w/v] in Mueller Hinton Broth), collected from three individual donors for time periods between 0 and 12 h. All three faecal samples had maximal binding of monensin (>90% binding) at 50% concentration. The results demonstrated the rapid and extensive binding of monensin to human faeces. An additional faecal interaction study was conducted, incorporating the microbiological and chemical assays. The mean proportions of monensin that became unavailable after 12 h interaction with faeces, as determined by the growth inhibition assay and chemical assay, were 96.8% and 94.3–98.6%, respectively. This confirmed the conclusions from the earlier study that the antibiotic activity of monensin in the colon would be reduced by >90% by contact with faecal material.

In terms of resistance development, monensin does not appear to select for true acquired resistance in bacteria and is not a drug used in human medicine. Results from the microbiological studies suggest that the development of resistance to monensin and cross-resistance to a number of commonly used antimicrobials in veterinary and human medicine is unlikely. Thus, the only potential adverse effect on human intestinal microbiota would be disruption of the colonization barrier as the end-point of concern for determining the microbiological ADI. However, since the majority of monensin residues in the colon are bound to faeces and are biologically inactive, the bioavailable concentration is below the lowest $MIC_{50}$ of any of the representative human intestinal bacteria. Therefore, monensin residues are unlikely to disrupt the colonization barrier of the human intestine. Consequently, there is no need to determine a microbiological ADI for monensin residues.

### *Evaluation*

Oral exposure to monensin results in skeletal and cardiac muscle damage and a decrease in leukocyte count and body weight gain. The effects on leukocytes and body weight gain occur at similar doses, which are lower than those that induce the effects in muscle. The effect on body weight gain was consistent, at similar doses, across studies in mice, rats and dogs; based on feed intake data in a rat study, it was considered to be a true treatment-related effect. The observed transient increase in coronary blood flow in dogs given a single oral dose of monensin was considered treatment related but not adverse, owing to the absence of effects on blood pressure or heart rate.

The Committee considered the effects of high doses of monensin on muscle tissue to be important adverse effects. The Committee also considered the consistent decrease in body weight gain at lower doses as a conservative indicator of monensin toxicity, even though the exact mechanism of this effect is not known. On the basis of the toxicological findings, the Committee selected the lowest relevant NOAEL of 1.14 mg/kg bw per day in the 2-year

oral rat study, based on a decrease in body weight gain at the next higher dose, as the basis for derivation of the ADI. The Committee noted that this NOAEL was supported by similar NOAELs for this effect in other species. An ADI of 0–10 μg monensin/kg bw was established by applying a safety factor of 100 to this NOAEL and rounding to one significant figure.

A toxicological monograph was prepared.

### *Residue evaluation*

#### *Data on pharmacokinetics and metabolism*

The fate of orally administered monensin has been studied in a number of different species.

In a study in cattle, calves received a single oral dose of 10 mg [$^{14}$C]monensin/kg bw in a gelatine capsule. Approximately 35% (in males) and 37% (in females) of the administered radioactivity were recovered in the bile; faecal excretion was the primary route of elimination. In additional studies, monensin or monensin metabolites were detected in plasma, liver and milk, demonstrating the absorption of monensin following oral administration.

Data are also available for pigs, sheep and goats. Again, monensin was eliminated rapidly, primarily in faeces. The metabolite profiles are qualitatively similar across all species. In all species, several metabolites were identified, but each represented less than 10% of the total residue.

Radiolabelled monensin was rapidly excreted in chickens, with approximately 75% of the administered dose eliminated in excreta within 3 days of dosing. Following intravenous administration, an elimination half-life of 2.11–5.55 h was calculated. Bioavailability following gavage administration was approximately 65%, and serum protein binding was approximately 23%. In turkeys, absorption was similar to that seen in chickens. The reported elimination half-life was 1.4–1.6 h.

Data from diverse species indicate that monensin is extensively metabolized. Monensin and monensin metabolites generally each represent small percentages (<10%) of the total residues. Monensin and monensin metabolites are found in faeces, urine, liver, bile, plasma and cows' milk.

#### *Residue data*

Radiolabelled total residue studies have been conducted in cattle, pigs, sheep, chickens and turkeys.

*Cattle.* In cattle, radiolabelled residues at zero withdrawal were highest in liver. Residue concentrations ranged from 0.21 to 0.59 mg equivalents/kg,

for dose equivalents of 33 and 44 mg/kg in feed, respectively. Total residues in other tissues were very much lower or non-detectable. In dairy cows treated by gelatine capsule (daily dose range: 900–1100 mg, corresponding to approximately 36 mg monensin/kg feed) for 9.5 days, tissue residues at zero withdrawal also were highest in liver (1.28 mg/kg) and much lower in other edible tissues.

Data from four unlabelled tissue residue studies in cattle were reviewed. In the earliest study, groups of cattle were fed monensin to provide 100, 500 or 750 mg/head per day. Monensin residue was detected only in one kidney of one animal in the highest dose group at zero withdrawal (bioautography sensitivity: 0.05 mg/kg). In a residue study in lactating dairy cows testing different routes of exposure, monensin was delivered by intraruminal controlled-release capsules (32 g monensin) and in a diet containing 24 mg monensin/kg (fed for 10 days) followed by a diet containing 36 mg monensin/kg (fed for 21 days). At zero withdrawal time, monensin was detected in four of six liver samples (residues ranged from 45.8 to 84.5 μg/kg). In another study, cows received monensin in feed (0, 24 or 36 mg/kg) or by gelatine capsule (1.8 mg/kg bw) through a rumen fistula. Liver was assayed at zero withdrawal, but there were no quantifiable residues (LOQ = 25 μg/kg).

In the most recent study (2007), lactating dairy cows were treated twice daily for 7 days with gelatine capsules to deliver 0.9 mg monensin/kg bw. Using an HPLC-MS/MS method (LOQ = 1.00 μg/kg), residues were determined at 6, 18 and 30 h after the final dosing. Mean residues in liver were 9.07 ± 1.86 μg/kg, 5.54 ± 0.81 μg/kg and 3.07 ± 1.57 μg/kg at 6, 18 and 30 h, respectively. Quantifiable residues were found in fat at 6 h and 18 h withdrawal (3.18 ± 2.08 μg/kg and 1.41 μg/kg, respectively) and in kidney at 6 h withdrawal (1.03 μg/kg). There were no quantifiable residues in muscle at any withdrawal time.

*Pigs.* Data from three radiolabelled studies in pigs were provided. In the first study, pigs received feed containing 55 mg monensin/kg. At all withdrawal times, liver had the highest concentration of radioactivity. Liver concentrations at zero withdrawal time were 1.67 mg monensin equivalents/kg (male) and 1.20 mg monensin equivalents/kg (female). The net radioactivity concentrations in other tissues were very low.

In a second radiolabelled study, pigs were dosed orally for 2.5 days at a level equivalent to 50 mg/kg in the feed. At slaughter 4 h after the final dosing, liver contained the highest net residue, 1.0–1.4 mg monensin equivalents/kg. Again, residues in the other tissues were low.

In a third study, six pigs were fed radiolabelled monensin (110 mg/kg in feed) for 5 days; samples were taken at 6 h (practical zero-time), 3 days and 5 days

of withdrawal. Livers from 6 h (practical zero withdrawal time) animals contained a mean of 2.3 mg equivalents total residue/kg. This decreased to a mean of 0.44 mg/kg by 5 days of withdrawal. Mean concentrations in kidney were 0.17 mg total residue/kg at zero withdrawal, decreasing to 0.05 mg/kg after 5 days. Concentrations in other edible tissues were uniformly low at all time points, less than 0.05 mg/kg. Monensin was not detected in the tissues of any of the treated animals at any time point using bioautography (sensitivity = 0.025–0.050 mg/kg) or HPLC (sensitivity 0.005 mg/kg) assays.

In an unlabelled residue study, pigs received a medicated feed containing 100 mg monensin/kg. No monensin residues were detected in any of the tissues assayed (bioautography; sensitivity of <0.05 mg/kg for muscle and <0.025 mg/kg for liver, kidney and fat).

*Sheep and goats.* Lambs were fed a complete ration containing 16.5 mg radiolabelled monensin/kg for 3, 5 or 7 dosing days. Mean liver total residues ranged from 0.20 to 0.36 mg monensin equivalents/kg at 12 h withdrawal ($n$ = 9). Parent monensin residues in liver were less than 0.05 mg/kg. Total residues in other tissues were less than 0.027 mg monensin equivalents/kg. There were no radiolabelled data provided for goats.

Residue data from studies using unlabelled monensin were provided for lambs and goats in separate studies. Lambs were fed a medicated ration containing 0, 11, 22 or 33 mg monensin/kg for 118 days and withdrawn for 0, 24 or 48 h. Residues were found only in liver at zero withdrawal (0.05–0.1 mg/kg) and 24 h withdrawal (below LOQ/sensitivity of 0.05 mg/kg), and not in muscle, fat or kidney. Goats were fed rations containing 0, 22 or 33 mg monensin/kg and withdrawn from medicated feed for 0 or 5 days. Residues were detected in liver samples of the low and high dose groups only at zero withdrawal, and only one sample was above the LOQ of 0.04 mg/kg. No monensin was detected in any of the 5-day withdrawal samples.

*Chickens and turkeys.* The distribution of radiolabelled residues at zero withdrawal has been extensively evaluated in chickens and turkeys. Chickens were treated with feed containing 110–125 mg [$^{14}$C]monensin/kg. In all the studies, liver was the edible tissue with the highest total residue at either a practical or true zero withdrawal (range = 0.434–0.935 mg/kg). Muscle had the lowest residue at zero withdrawal (range = 0.006–0.059 mg/kg). Residues in fat and skin/fat depleted slowly, but the highly persistent radioactivity was attributed to incorporation of label into endogenous fatty acids. At 3 days of withdrawal, concentrations of radiolabelled residues in liver had depleted to below the concentrations of radiolabelled residues in fat and skin. Unextracted radioactivity in liver increased from 38% to 69% during the 5-day withdrawal, even as the total liver radioactivity decreased.

In turkeys treated with [$^{14}$C]monensin at 110 mg/kg feed for 5 days, liver had the highest mean radioactive residue, and muscle had the lowest (0.91 mg/kg and <0.03 mg/kg, respectively), paralleling the findings in chickens.

A series of non-radiolabelled studies have been conducted in chickens and turkeys. In the first study, chickens were preconditioned on feed containing 120 mg monensin/kg for 45 days and then transferred to feed containing 16.5, 50 or 120 monensin/kg for 5 days. At zero withdrawal, monensin concentrations below 0.04 mg/kg were detected in fat samples from birds in the 120 mg monensin/kg group. No other tissues or treatment groups had detectable monensin residues. In another study, chickens were fed monensin (120 mg monensin/kg) alone or in combination with other feed additives. Although a few samples (out of more than 2000 analysed) were positive at 0 and 24 h withdrawal, samples from chickens withdrawn for 48, 72 and 96 h were all negative for monensin residues. In another study, chickens received medicated feed containing 80, 100 or 120 mg monensin/kg. Concentrations of monensin residues at zero withdrawal (bioautography; sensitivity 0.025 mg/kg) were 0.057–0.110 mg/kg in fat, undetectable to 0.035 mg/kg in muscle, undetectable to 0.039 mg/kg in liver and undetectable to 0.014 mg/kg in kidney. No detectable residues of monensin were found in fat (48 h or more withdrawal) or in liver, muscle and kidney (24 h or more withdrawal). When broiler chickens received monensin at 40 mg/kg bw by gavage, the highest concentrations of monensin residues were found in liver (2.4 ± 0.06 mg/kg at 2 h withdrawal). Detectable residues were found in liver, kidney and fat at 24 h withdrawal and in liver at 48 h withdrawal (0.13 ± 0.02 mg/kg). In a recent study (2007), chickens were treated with 125 mg monensin/kg feed for 42 days. At zero withdrawal, the highest concentration of monensin was found in skin with fat (24.3 μg/kg), followed by liver (14.8 μg/kg) and kidney (14.2 μg/kg). No monensin residues were detected in muscle samples. There were no detectable residues in any tissues collected at 12 or 48 h withdrawal. In another recent study (2007), chickens were treated orally with monensin (121 mg monensin/kg feed). Residues, determined using a sensitive LC-MS/MS method, declined rapidly and were observed only in fat at the 18-h sampling. Unpublished data with close-interval (every 2 h) sample collections show that monensin residues through the 12-h sampling point are highest in fat, followed by liver.

Two residue depletion studies with unlabelled monensin were provided for turkeys. In the first study, turkeys were fed a medicated feed containing 120 mg monensin/kg. Detectable residues (bioautography; limit of sensitivity = 0.05 mg/kg) were found in all tissues at zero withdrawal, in fat and kidney samples through 24 h withdrawal, and in muscle and skin to 48 h post-treatment. Liver samples were negative at 72 h. In a recent unpublished study (2008), turkeys received monensin at a dose rate of 100 mg/kg in feed. Close-interval sample collections show that monensin residues through the 24-h sampling point are highest in fat, followed by liver.

*Quail.* Quail were reared for 8 weeks on a diet containing 80 mg monensin/kg. At the end of the feeding period, the birds were slaughtered, with no withdrawal period. No monensin was detected in any of the liver samples (composites from 5–6 birds) from monensin-treated birds using a thin-layer bioautographic method (LOQ = 0.04 mg/kg).

*Cows' milk.* Total residues in cows' milk reached steady state (average concentration = 45 μg/kg) within 5 days when cows were dosed by gelatine capsules (1.8 mg/kg bw). Parent monensin was detected at concentrations below 1 μg/kg. Approximately 26% of the radioactivity contained in milk was attributed to incorporation into endogenous fatty acids, rather than from monensin-related residues.

Three milk residue depletion studies using unlabelled monensin were provided. In one study, lactating dairy cows (weight range: 580–725 kg) were treated with two controlled-release capsules (32 g monensin per capsule; release rate range: 408.5–469.9 mg/day) and fed a medicated feed containing 24 mg monensin/kg (for 10 days) followed by medicated feed containing 36 mg monensin/kg (for 21 days) (an estimated average daily dose of 1800 mg monensin per cow). While on treatment, there were no detectable residues of monensin in milk (<5 μg/kg). In another study, Holstein cows received monensin in feed (0, 24 or 36 mg/kg) or with gelatine capsules (1.8 mg/kg bw) through a rumen fistula. None of the milk samples at any of the three milkings sampled contained residues of monensin at or above the LOQ of the HPLC-UV method (0.005 mg/kg). In the most recent study (2007), lactating dairy cows were treated twice daily (total daily dose: 0.9 mg monensin/kg bw) for 7 days with gelatine capsules. All of the samples collected at the first milking contained quantifiable monensin residues (range: 0.54–0.32 μg/kg). Only one sample collected at the second milking (0.32 μg/kg) and no samples collected at the third milking contained quantifiable monensin residues (LOQ = 0.25 μg/kg).

### *Analytical methods*

The earliest semiquantitative method for the analysis of monensin in animal tissues and fluids was based on thin-layer chromatography with bioautography. It has a sensitivity of 0.025 mg/kg and a routine performance limit of 0.05 mg/kg. It has been further refined to have a sensitivity limit of 0.01 mg/kg.

An HPLC method with post-column derivatization using vanillin and detection at 520 nm has been validated for poultry and bovine tissues and cows' milk. The LOQ is 0.025 mg/kg for tissues and 0.005 mg/kg for milk.

More recently (2004), a method utilizing extraction with an organic solvent and cleanup on SPE columns followed by LC-MS/MS using $C_{18}$ columns and ESI methods has been developed. This method is suitable for the determination of monensin residues in whole eggs and in bovine, porcine and avian

tissues, including muscle, liver and fat, with a decision limit (CCα[1]) of 0.2 μg/kg. A validated HPLC-MS/MS method (internal standard: narasin) is available for use in the analysis of chicken tissues and plasma. Most recently (2007), a validated HPLC-MS/MS method with ESI-MS/MS was developed in accordance with GLP and European guidelines (*15*). Using liquid-phase extraction and a narasin internal standard, separation is achieved with a reversed-phase column and gradient elution. The method LOQ is 1.00 μg/kg for tissues and 0.25 μg/kg for milk.

*Maximum residue limits*

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

- An ADI of 0–10 μg/kg bw was established by the Committee based on a chronic toxicological end-point. This ADI is equivalent to up to 600 μg monensin for a 60-kg person.
- Monensin is the marker residue in both tissues and milk.
- Monensin is extensively metabolized; monensin represents, conservatively, 5% of total residues in tissues and 2.7% in milk.
- Liver contains the highest concentration of total residues at zero withdrawal in all species tested. In chickens treated at the maximum dose of 125 mg/kg in feed, total residues in abdominal fat exceed those in liver at 3 and 5 days of withdrawal. Liver can serve as the target tissue.
- While residue data in the studies submitted were determined using several methods, newer methods include a validated HPLC method with post-column derivatization and a validated HPLC-MS/MS method. Both of these newer methods are suitable for routine monitoring.
- The MRLs recommended for poultry tissues were based on residue data from the unlabelled residue depletion studies. For cattle, the residue concentrations were determined using the validated HPLC method with post-column derivatization. For chickens and turkeys, the residue concentrations were determined using the validated HPLC-MS/MS method.
- The MRL recommended for cows' milk was based on unlabelled residue depletion data determined using the validated HPLC-MS/MS method. The recommended milk MRL is 8 times the LOQ (0.25 μg/kg) for that method.

[1] CCα means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

— Because monensin is not currently approved for use in pigs, no MRLs were recommended for monensin residues in pig tissues.

The Committee recommended permanent MRLs for monensin in poultry (chicken, turkey and quail) tissues of 10 μg/kg in liver, kidney and muscle and 100 μg/kg in fat. The Committee recommended permanent MRLs for monensin in ruminant (cattle, sheep and goat) tissues of 10 μg/kg in kidney and muscle, 20 μg/kg in liver, 100 μg/kg in fat and 2 μg/kg in milk. Residues in all species are determined as monensin.

It was not possible to do an intake estimate for monensin owing to the small number of residue data points. Using the model diet and marker to total residue ratios of 5% for tissues and 2.7% for milk, the MRLs recommended above would result in an intake of 301 μg/person per day (poultry tissues plus milk) or 321 μg/person per day (ruminant tissues plus milk), which represent 50% and 54% of the upper bound of the ADI, respectively.

A residue monograph was prepared.

## 3.6 Narasin

### *Explanation*

Narasin belongs to the polyether monocarboxylic acid class of ionophores produced by *Streptomyces aureofaciens* strain NRRL 8092. Narasin is composed of 96% narasin A, 1% narasin B, 2% narasin D and 1% narasin I. Narasin A has the major activity (85%). The biological activity of narasin is based on its ability to form lipid-soluble and dynamically reversible complexes with cations. Narasin functions as a carrier of these ions, mediating an electrically neutral exchange–diffusion type of ion transport across membranes. The resultant changes in transmembrane ion gradients and electrical potentials produce critical effects on cellular function and metabolism of coccidia. Narasin is effective against sporozoites and early and late asexual stages of coccidia infections in broiler chickens, caused by *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix* and *E. tenella.* Narasin is also used for the prevention of necrotic enteritis in broiler chickens. In cattle, narasin is used as a growth promoter. Narasin's growth-promoting effect is attributed to shifts in the ruminal bacterial population and subsequent modulation of rumen fermentation.

The antimicrobial spectrum of activity of narasin is limited mainly to Gram-positive bacteria, including *Enterococcus* spp., *Staphylococcus* spp. and *Clostridium perfringens*. Narasin is not used in human medicine and was therefore not classified as a critically important antibiotic for humans by the 2007 WHO expert meeting on categorization of critically important

antimicrobials for human medicine for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use (*14*).

Narasin has not been previously reviewed by the Committee. Narasin was evaluated on priority request by the 17th Session of CCRVDF (*7*). The Committee was requested to establish an ADI and recommend MRLs for narasin residues in chicken, pigs and cattle.

The Committee considered the results of studies on pharmacokinetics and metabolism, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive and developmental toxicity, general pharmacology, microbiological safety and exposures in humans. Studies were conducted with the dried biomass form of narasin referred to as mycelial narasin or the purified crystalline form of narasin. Mycelial narasin is used in the manufacture of narasin products used in food-producing animals.

### *Toxicological and microbiological evaluation*

#### *Biochemical data*

Owing to the principal effect of narasin on the microflora of the gastrointestinal tract (including coccidia), few conventional pharmacokinetic studies have been performed. Studies in both target and laboratory animals indicate that narasin depletes quickly in plasma. Narasin is metabolized by the liver by oxidation/hydroxylation pathways, resulting in polar metabolites that are readily excreted in bile. The primary route of excretion of narasin is in the faeces, with total elimination occurring within 3–4 days.

The metabolism and excretion of narasin were evaluated in a series of studies in rats. Following a single oral dose of 2.3 mg of [$^{14}$C]narasin, 75% of the total radioactivity was recovered in the urine and faeces of rats by 52 h post-dosing. Only 1.1% of the total excreted radioactivity was found in the urine, and the remainder (98.9%) was in the faeces. In a second study, more than 16 structural isomer metabolites of narasin and narasin B were identified in the faeces using HPLC/ion spray mass spectrometry (ISP-MS). In another study, it was concluded that [$^{14}$C]narasin was metabolized by rats to more than 20 metabolites, and the patterns in faeces and liver were qualitatively similar.

#### *Toxicological data*

Mycelial and crystalline forms of narasin were tested for toxicity. Doses of both forms of narasin in the toxicological studies were reported on the basis

of the microbiological activity of narasin relative to that of the crystalline form.

The acute oral toxicity of narasin varies between species, being extremely toxic (defined as an $LD_{50}$ of 1 mg/kg bw or less) to horses, highly toxic (defined as an $LD_{50}$ of 1–50 mg/kg bw) to pigs, mice, rats and rabbits, and moderately toxic (defined as an $LD_{50}$ of 50–500 mg/kg bw) to chickens and quail. The primary clinical signs of acute toxicity following an oral dose of mycelial or crystalline narasin were hypoactivity, leg weakness and ataxia.

In a non-GLP-compliant 3-month oral toxicity study, mice (15 per sex per group) were fed mycelial narasin at 0, 10, 20 or 40 mg narasin activity/kg in the diet, equivalent to 0, 1.5, 3 and 6 mg narasin activity/kg bw per day. There were no toxicologically relevant changes in survival, clinical signs, body weight or haematological, clinical chemistry or anatomic pathology. The NOAEL was 40 mg/kg in the diet, equivalent to 6 mg narasin activity/kg bw per day, which was the highest dose tested.

In a second 3-month non-GLP-compliant oral toxicity study, mice (15 per sex per group) were fed mycelial narasin at levels of 0, 60, 80 or 100 mg narasin activity/kg in the diet, equivalent to 0, 9, 12 and 15 mg narasin activity/kg bw per day. At termination, mean body weights of males in the 80 and 100 mg/kg dietary groups were decreased by 26% and 27%, respectively. The mean body weight of females in the 100 mg/kg dietary group was decreased by 14%. On the basis of the magnitude of the body weight changes at 80 and 100 mg/kg in the diet, the NOAEL was 60 mg/kg in the diet, equivalent to 9 mg narasin activity/kg bw per day.

In a 3-month non-GLP-compliant oral toxicity study, rats (15 per sex per group) were fed mycelial narasin at levels of 0, 15, 30 and 60 mg narasin activity/kg in the diet, equal to 0, 1.1, 2.2 and 4.7 narasin activity/kg bw per day for males and 0, 1.1, 2.6 and 5.7 mg narasin activity/kg bw per day for females. Decreases in feed conversion ratios, mean body weight and mean body weight gain were adverse (exceeding 10% of control values) narasin-related effects seen in males and females at the 30 and 60 mg/kg dietary levels. The decreased mean body weight in females in the 60 mg/kg dietary group was statistically significant. On the basis of decreased feed conversion ratios, mean body weight and mean body weight gain, the NOAEL was 15 mg/kg in the diet, which was equal to 1.1 mg narasin activity/kg bw per day.

In a 1-year non-GLP-compliant oral toxicity study, rats (15 per sex per group) were fed mycelial narasin at levels of 0, 7.5, 15 or 30 mg narasin activity/kg in the diet, equal to 0, 0.49, 1.0 or 1.9 mg narasin activity/kg bw per day for males and 0, 0.57, 1.2 or 3.2 mg narasin activity/kg bw per day for females. Decreases in mean body weight and feed conversion ratios relative to control

in females in the 30 mg/kg dietary group and decreased feed conversion ratios in females in the 15 mg/kg dietary group were attributed to unpalatability and spillage of the diet. This was evidenced by a significant decrease in mean food consumption and mean body weight loss in females in the 30 mg/kg dietary group in week 1, followed by significantly increased weekly mean food consumption in most weeks thereafter. Non-significant increases in weekly mean food consumption by females in the 15 mg/kg dietary group were also observed. Therefore, the effects on mean body weight and feed conversion ratio are not toxicologically relevant findings. There were no treatment-related effects in males or females at any dose level. In the absence of evidence of toxicity, the NOAEL was 30 mg narasin activity/kg in the diet, equal to 1.9 mg narasin activity/kg bw per day, which was the highest dose tested.

In a 3-month non-GLP-compliant oral toxicity study, dogs (four per sex per group) were given mycelial narasin in gelatine capsules at dose levels of 0, 0.5, 1.0 or 2.0 mg narasin activity/kg bw per day. Clinical signs of leg weakness and incoordination at 2.0 mg/kg bw per day were attributed to treatment with narasin. The electrocardiograms (ECGs) of six of the eight 2.0 mg/kg bw per day animals showed decreased heart rate, with normal to markedly abnormal sino-arrhythmias present. Three of these dogs had signs of sino-arrest. No histological lesions were found in the hearts of these same animals, and there were no corresponding changes in blood chemistry values. On the basis of clinical signs of leg weakness, incoordination and abnormal ECG findings seen at 2.0 mg/kg bw per day, the NOAEL was 1.0 mg narasin activity/kg bw per day.

To further define the signs of toxicity and evaluate the effects of narasin on ECGs in dogs, a non-GLP-compliant 6-month study was conducted in dogs (four per sex per group) given mycelial narasin orally by gelatine capsule at dose levels of 0, 0.5, 1.0 or 1.5 mg narasin activity/kg bw per day before the morning feeding. Abnormal ECG findings consisting of bradycardia, low-amplitude R-waves, elevated T-waves and marked suppression of the ST segment were seen in a single 1.5 mg/kg bw per day female at the 1-month interval. These findings were deemed treatment related, because treatment-related effects on ECGs were seen in a separate 3-month oral toxicity study of narasin in dogs. However, ECG changes in this animal were absent at the 3- and 6-month ECG examinations, providing evidence of reversibility, and there was no evidence of microscopic changes in the heart. On the basis of the reversible abnormal ECG findings, the NOAEL was 1.0 mg narasin activity/kg bw per day.

In a GLP-compliant study, male and female dogs (four per sex per group) were given daily oral doses of mycelial narasin by gelatine capsule at 0, 0.5,

1.0 or 2.0 mg narasin activity/kg bw per day for 1 year. An additional group (four per sex) was given crystalline narasin orally by capsule at a dose level of 2.0 mg/kg bw per day. One male in the 2.0 mg/kg bw per day mycelial group was found moribund, with clinical signs of anorexia, excessive salivation, laboured respiration and recumbency, and was killed on day 13. Clinical signs and microscopic lesions observed in the heart indicated that the moribund condition of this animal was treatment related. Effects related to treatment with mycelial narasin at 2.0 mg/kg bw per day in all surviving animals were slight to severe leg weakness, excessive salivation, loss of muscle tone and patellar reflex deficits. Less frequently observed effects were convulsions or opisthotonos, bilateral dropped carpus, transient occurrences of tremors, decreased food consumption, hypoactivity and laboured respiration. Effects related to treatment with crystalline narasin at 2.0 mg/kg bw per day were of lower incidence or severity and included leg weakness, ataxia, excessive salivation, depressed appetite, laboured respiration, decreased muscle tone and patellar reflex deficits. Effects related to treatment in the 1.0 mg narasin activity/kg bw per day mycelial narasin group included transient leg weakness and slight muscle atrophy observed clinically, but not histologically, in one male and occasional salivation in two females. Decreases in body weight gain were observed in the 2.0 mg/kg bw per day mycelial and crystalline narasin groups, but were of greater magnitude in the mycelial narasin group. Mean body weight was decreased in males (11% and 15%) and females (29% and 11%) in the 2.0 mg/kg bw per day mycelial and crystalline narasin groups, respectively. These decreases were associated with decreases of 10% or greater in monthly mean food consumption at multiple intervals during the study. There were no treatment-related ECG findings. Transient increases in creatine phosphokinase and AST values and degenerative and/or regenerative changes in cardiac and skeletal muscles and sciatic, tibial and other unspecified peripheral nerves were narasin-related effects seen in animals given mycelial or crystalline narasin at 2.0 mg/kg bw per day. The histopathological changes were more severe in the mycelial narasin group. Heart muscle lesions were observed only in the 2.0 mg/kg bw per day dog that was killed in extremis. On the basis of mortality, clinical signs, effects on body weight, clinical chemistry and microscopic findings in skeletal and cardiac muscles and peripheral nerve, the NOAEL was 0.5 mg narasin activity/kg bw per day.

In replicate 2-year, GLP-compliant combined toxicity/carcinogenicity studies, mice (30 per sex per group per replicate) were fed mycelial narasin at 0, 5, 15 or 50 mg/kg of the diet, equal to 0, 0.59, 1.91 and 7.16 mg narasin activity/kg bw per day for males and 0, 0.71, 2.29 and 8.72 mg narasin activity/kg bw per day for females. An additional group of 30 mice per sex was fed crystalline narasin at 50 mg/kg in the diet, equal to 8.24 and 9.49 mg/kg bw per day for males and females, respectively. Mice tolerated these

levels of exposure with no treatment-related effects on survival and no evidence of carcinogenicity. Decreases in mean body weight and body weight gain were observed for males and females at mycelial and crystalline narasin dietary levels of 15 and 50 mg/kg. Body weight decreases in the 50 mg/kg dietary crystalline narasin group were greater than those in the 50 mg/kg dietary mycelial narasin group. In both replicates, on the basis of effects on body weight, the NOAEL was 5 mg narasin activity/kg in the diet, equal to 0.59 narasin activity/kg bw per day.

In rats, two non-GLP-compliant 2-year combined chronic toxicity/carcinogenicity studies were conducted. The first study had 60, 40, 40 and 40 rats per sex per group, and the second study had 60, 40, 40 and 39 males:41 females per sex per group, for the control, low dose, middle dose and high dose groups, respectively. In both studies, rats were fed mycelial narasin for 2 years at 0, 7.5, 15 or 30 mg narasin activity/kg of the diet, equal to 0, 0.31, 0.59 and 1.15 mg narasin activity/kg bw per day for males and 0, 0.38, 0.76 and 2.0 mg narasin activity/kg bw per day for females in the first study and 0, 0.29, 0.62 and 1.26 mg narasin activity/kg bw per day for males and 0, 0.37, 0.88 and 2.34 mg narasin activity/kg bw per day for females in the second study. In both studies, the rats tolerated these levels of exposure with no treatment-related effects on survival and no evidence of carcinogenicity. The only clinical finding clearly related to treatment with narasin was decreased body weight gain in females at the 30 mg/kg dietary narasin level. On the basis of this body weight effect, the NOAEL was 15 mg narasin activity/kg diet, equal to 0.76 mg narasin activity/kg bw per day in the first study and 0.88 mg narasin activity/kg bw per day in the second study.

Crystalline narasin produced negative results in an adequate range of in vitro and in vivo genotoxicity studies. The Committee concluded that narasin had no genotoxic potential.

In a non-GLP-compliant multigeneration reproductive toxicity study, rats (45 per sex per group for the parental generation and 25 per sex per group in subsequent generations) were fed mycelial narasin at levels of 0, 7.5, 15 and 30 mg narasin activity/kg in the diet, equal to 0, 0.8, 1.8 and 3.8 mg narasin activity/kg bw per day during the growth phases. A total of four generations, three parental generations and their offspring, were exposed to narasin. Narasin was well tolerated, with no evidence of reproductive impairment or effect on the progeny when fed at levels of 0, 7.5 and 15 mg/kg of the diet. At the 30 mg/kg dietary level, parental female body weight was generally lower than control, and mean progeny body weights were significantly decreased. However, the reproductive capacity of the rats was normal. The NOAEL based on effects on body weight in parents and offspring was 15 mg narasin activity/kg of the diet, equal to 1.8 mg narasin activity/kg bw per day.

The NOAEL for reproductive effects was 30 mg narasin activity/kg of the diet, equal to 3.8 mg narasin activity/kg bw per day, which was the highest dose tested.

The teratogenicity of mycelial narasin in rats was evaluated as a segment in each of the three generations from the aforementioned multigeneration reproductive toxicity study. Rats (22–25 per sex per group) were fed narasin at levels of 0, 7.5, 15 or 30 mg narasin activity/kg in the diet, equivalent to 0, 0.5, 1.3 or 3.5 mg narasin activity/kg bw per day. Pregnant females were killed on gestation day 20. Maternal mean body weight at the 30 mg/kg dietary level was significantly decreased compared with control. The NOAEL for maternal toxicity was 15 mg narasin activity/kg in the diet, equal to 1.3 mg narasin activity/kg bw per day. The NOAEL for teratogenicity was 30 mg narasin activity/kg in the diet, equal to 3.5 mg narasin activity/kg bw per day, the highest dose tested.

Pregnant rabbits (15 per dose) were dosed by oral gavage with mycelial narasin at dose levels of 0, 0.6, 1.2 or 1.8 mg narasin activity/kg bw per day. Maternal toxicity occurred at 1.2 and 1.8 mg narasin activity/kg bw per day and included mortality, abortions, leg weakness and incoordination. Non-significant decreases in mean body weight and food consumption relative to control were also noted at these dose levels. Non-significant decreases in mean live litter size and a slight increase in resorptions relative to control were noted at 1.8 mg/kg bw per day. There was no evidence of teratogenicity. The NOAEL for maternal toxicity was 0.6 mg narasin activity/kg bw per day. The NOAEL for teratogenicity was 1.8 mg narasin activity/kg bw per day, which was the highest dose tested.

A review of the medical records and incident/injury reports for 163 employees occupationally exposed to narasin indicated two cases of confirmed IgE-mediated allergic responses to narasin, which were manifested by transient facial uticaria, pruritis, nasal congestion and chest tightness. Three additional cases with similar respiratory symptoms were not confirmed by intradermal testing and might have represented local respiratory irritation rather than an allergic response. After relocation away from the narasin manufacturing area, the symptoms in all five cases resolved completely, with no long-term consequences.

### *Microbiological data*

The JECFA decision tree approach that was adopted by the sixty-sixth Committee (Annex 1, reference *181*) and complies with VICH GL36 (*8*) was used to evaluate the impact of narasin residues on the intestinal microflora.

Narasin activity was clearly demonstrable against *Bifidobacterium*, *Clostridium*, *Enterococcus*, *Eubacterium*, *Lactobacillus*, *Fusobacterium* and *Peptostreptococcus*. *Peptostreptococcus* was the most susceptible group, with a MIC range of 0.062–0.5 μg/ml and a $MIC_{50}$ of 0.125 μg/ml. Narasin exerted very weak activity against *Bacteroides fragilis* and other *Bacteroides* spp., with a $MIC_{50}$ of 32 μg/ml. Narasin exerted no measurable antibacterial activity against *E. coli* (MIC >128 μg/ml). Therefore, the only potential adverse effect on human intestinal microbiota would be disruption of the colonization barrier as the end-point of concern for determining the microbiological ADI. However, since the majority of narasin residues in the colon are bound to faeces (99.4%) and biologically inactive, the bioavailable concentration is below the $MIC_{50}$ of 0.125 μg/ml for *Peptostreptococcus*, which is the most sensitive genus of the representative human intestinal bacteria. In addition, to study the ability of narasin to affect faecal excretion of *Salmonella* in poultry, broiler chickens were fed narasin at 100 mg/kg of the diet and the next day dosed with *Salmonella* for periods up to 8 weeks. Narasin treatment had no statistically significant effect on the faecal excretion of *Salmonella* or on the proportion of faecal coliforms resistant to narasin. Therefore, narasin did not affect the normal intestinal microbiota. Based on MIC susceptibility, faecal binding interaction and in vivo colonization barrier studies, narasin residues were considered unlikely to disrupt the colonization barrier of the human gastrointestinal tract. Narasin does not appear to select for acquired resistance in bacteria, as there is no genetically encoded resistance gene for ionophores. The Committee concluded that it was not necessary to determine a microbiological ADI for narasin.

### *Evaluation*

The lowest NOAEL was established in the GLP-compliant, 1-year oral toxicity study in the dog. The NOAEL in this study was 0.5 mg/kg bw per day, based on clinical and pathological signs of toxicity at the higher doses. Safety factors of 10 for interspecies differences and 10 for interindividual variability in the population were applied, for a total safety factor of 100. This safety factor was considered appropriate in view of the extensive database of adequately conducted studies for a compound that has a long history of use. Therefore, an ADI of 0–5 μg/kg bw was established on the basis of the toxicological data.

A toxicological monograph was prepared.

### *Residue evaluation*

#### *Data on pharmacokinetics and metabolism*

*Chickens.* The pharmacokinetics of narasin in chickens was evaluated in several studies. Narasin was rapidly cleared from plasma and tissues.

Three studies were conducted to evaluate the metabolism of narasin in chickens. In one study, radiolabelled narasin was extensively metabolized by chicken liver, with oxidation serving as the primary pathway of metabolism. Narasin A metabolites represented 88.9% of total radioactivity, and more than 85% of the dose was recovered in excreta within 48 h. Liver was the tissue with the highest concentration of radioactivity, followed by fat, kidney and muscle. At least 15 metabolites and parent narasin were identified from the excreta. The distribution and relative magnitude of radioactivity from liver and excreta were similar. Narasin metabolites were 20 times less active than parent narasin.

*Cattle.* Two studies were evaluated. In one study, following oral administration (approximately 79.2 mg/day, by gelatine capsule) of [$^{14}$C]narasin, a total of 91% of the administered radioactivity was recovered, 89% in the faeces and 2% in the urine. Liver was the only edible tissue that contained appreciable concentrations of residue. Little more than trace concentrations of residues were found in the other tissues. The most abundant metabolite in cattle liver was NM-12, narasin monohydroxylated in ring B. It represented approximately 15% of the liver radioactivity. Parent narasin represented approximately 5% of the total residue.

*Pigs.* Two GLP-compliant studies were conducted. Following oral administration of [$^{14}$C]narasin in feed, approximately 90% of the administered radioactivity was recovered in the faeces, and less than 10% in the urine. The metabolite profile was qualitatively similar to that seen in cattle, and liver was the only edible tissue that contained appreciable concentrations of residue.

#### *Residue data*

*Cattle.* In a GLP-compliant study, six steers and three heifers received a single capsule containing [$^{14}$C]narasin equivalent to 13 mg/kg in feed. The animals were dosed twice daily for 5 consecutive days. At all slaughter times, liver contained the highest concentrations of radioactivity, corresponding to 0.49, 0.23 and 0.05 mg narasin equivalents/kg at zero withdrawal time, 1 day and 3 days, respectively. Less than 5% of the liver radioactivity corresponded to parent narasin. Muscle, fat and kidney contained less than 0.02 mg narasin equivalents/kg at zero withdrawal. In a non-GLP-compliant study with

non-radiolabelled narasin, 18 cattle were fed 150 mg narasin/head per day (66 mg/kg in diet) for 140 days. Residues were found in the fat and liver until 48 h post-withdrawal (≤10–20 μg/kg at zero withdrawal, ≤5–10 μg/kg at 24 and 48 h withdrawal) (thin-layer bioautographic method sensitivity = 5 μg narasin/kg). Less than 5 μg narasin/kg was found in the muscle tissue at zero withdrawal, and no residues were found at 24 h withdrawal time. No residues were found in kidney at any time.

*Pigs.* In a GLP-compliant study, 12 pigs were fed with a ration containing [$^{14}$C]narasin at a level equivalent to 37.5 mg/kg feed for 5 days. The mean total residues in liver for 0, 24, 48 and 72 h withdrawal were 0.51, 0.44, 0.26 and 0.18 mg/kg, respectively. Muscle and kidney contained no detectable radioactive residues at zero withdrawal, and fat contained less than 0.05 mg narasin/kg. The analytical method was a thin-layer chromatography–bioautographic method, using *Bacillus subtilis* as the indicator organism. In a non-GLP-compliant study, 24 pigs were fed ad libitum for 14 days with a finishing ration containing 0 or 45 mg narasin/kg in feed. Tissues were collected at 12 and 24 h withdrawal time. No residues at or above the LOQ of the HPLC-UV method (25 μg/kg) were observed in the tissues of any animals slaughtered at either time point.

*Chickens.* Two residue depletion studies with [$^{14}$C]narasin were evaluated in chickens. In a non-GLP-compliant study, nine chickens were preconditioned on a ration containing a nominal 80 mg narasin/kg feed. At about 8 weeks of age, the birds were dosed with 80 mg [$^{14}$C]narasin ration/kg ad libitum for 5 days and then slaughtered at 0, 1 and 3 days of withdrawal. Liver contained the highest $^{14}$C residues, and muscle contained the lowest. At 3 days of withdrawal, all residues were below 25 μg/kg with the exception of liver, which had residue concentrations of approximately 65 μg/kg. In a GLP-compliant study, 15 broiler chickens were dosed for 5 days with a broiler ration containing 100 mg [$^{14}$C]narasin/kg. Liver, kidney, fat, muscle and skin samples were taken. The zero withdrawal time mean residues of narasin in milligram per kilogram equivalents were as follows: liver, 0.45; fat, 0.21; skin, 0.14; kidney, 0.14; and muscle, 0.02. Following withdrawal of medication, the radiochemical residues depleted rapidly in all tissues. After a 1-day withdrawal, the residue concentrations had declined by more than 50%, and all tissues except liver (0.18 mg/kg) were below 0.1 mg/kg.

In a GLP-compliant comparative metabolism study in cattle, dogs and rats, radiochemical residues were extracted from liver and faeces. Metabolite patterns in all three species were qualitatively similar. It was shown in this study that the toxicological test animals, rat and dog, were exposed to the same metabolites following oral dosing with narasin. In a metabolite study in rats dosed orally with narasin, faeces were examined to determine specific narasin

metabolism in the rat. The results of this study showed that the same pathways (oxidation/hydroxylation) for metabolism in the chicken are shared by the rat, and hydroxylated metabolites found in chickens were tested by autoexposure in rat toxicity studies with narasin.

Three GLP-compliant studies were conducted to evaluate depletion of unlabelled narasin in chickens. In the first study, 32 broiler chickens were fed 80 mg narasin/kg in feed for 5 consecutive days. Tissue samples were taken at 0, 6, 12 and 24 h withdrawal. Narasin was quantified by HPLC with UV detection after post-column derivatization (LOQ = 25 μg/kg; LOD = 0.6 μg/kg). Narasin was not detected in muscle or kidney at any of the slaughter times. In liver, narasin was detected only in the 6-h withdrawal sample. Narasin was detected in skin/fat at 0 and 6 h withdrawal. No residues were detected in any tissues beyond 12 h withdrawal. In the second study, broiler chickens were fed from day 0 to day 42 with a medicated ration containing either 80 or 160 mg narasin/kg. Nine chickens per group were slaughtered at 2, 24, 72, 120 and 168 h withdrawal. Narasin analyses were conducted by bioautography using *Bacillus stearothermophilus* var. *calidolactis* C-953 as the indicator organism (LOQ = 25 μg/kg). In the 80 mg/kg dose group, narasin residues were quantified in fat and skin at 2 and 24 h after treatment. In muscle, liver and kidney, no quantifiable narasin residues were found. In the 160 mg/kg dose group, narasin residues were quantified in skin up to 24 h and in fat up to 2 h. There were no quantifiable residues in muscle, liver or kidney at any sampling time. In the third study, 80 broiler chicks were fed for at least 45 days with a ration containing 80 mg narasin/kg. Birds were slaughtered after 6, 12, 18 and 28 h withdrawal. Residues were determined using a bioautographic assay using *Bacillus subtilis* as the indicator organism. The LOQ was 5 μg/kg. Mean residue concentrations ranged from 86.1 ± 50.6 μg/kg (fat) and 58.8 ± 14.9 μg/kg (skin) at 6 h withdrawal to 13.0 ± 9.5 μg/kg (fat) and 8.2 ± 2.6 μg/kg (skin) at 28 h withdrawal.

### *Analytical methods*

*Screening methods.* Screening by thin-layer chromatography–bioautography using *Bacillus stearothermophilus* var. *calidolactis* C-953 inoculum has been developed for detection of narasin with an LOQ of 25 μg/kg in edible tissues. The recoveries range from 84% to 100%. A second thin-layer chromatography–bioautographic method, using *Bacillus subtilis* as the indicator organism, with an LOQ of 5 μg/kg, was also reported. In depletion studies conducted in cattle, residues were determined using a thin-layer bioautographic method with a limit of sensitivity of 5 μg narasin/kg.

*Confirmatory methods.* Available analytical methods for the determination of narasin residues include HPLC-UV and HPLC-MS/MS. The HPLC

method with UV detection has been validated for poultry and pig tissues. The LOQ is 25 μg/kg for tissues. An improved method for use with chicken tissues has an LOQ of 7 μg/kg. Mass spectrometric methods provide enhanced sensitivity, with an LOQ of 1 μg/kg (one study) and CCα[1] of 0.3 or 1.6 μg/kg (two additional studies). Both the HPLC-UV and the HPLC-MS/MS methods are considered suitable for monitoring purposes. As a result, in recommending MRLs for pigs, chickens and cattle, the Committee used the LOQ values for the HPLC-UV method. The method has not been fully validated for cattle tissues.

*Maximum residue limits*

In recommending MRLs for narasin in chickens and pigs and temporary MRLs for cattle, the Committee considered the following factors:

- An ADI of 0–5 μg/kg bw was established by the Committee based on a toxicological end-point. This ADI is equivalent to up to 300 μg for a 60-kg person.
- Narasin A is considered a suitable marker residue in tissue.
- Metabolites exhibit little or no microbiological activity in vitro. Unchanged narasin represents approximately 5% of the total residues in liver.
- Liver contains the highest concentrations of residues. In fat, narasin residues persist for up to 72 h. For this reason, liver or fat (skin/fat in natural proportions, where applicable) are considered suitable choices for the target tissue.
- Residue data in the studies submitted were determined using several methods. These methods include a validated HPLC method with post-column derivatization and UV detection and a validated HPLC-MS/MS method. Both of these newer methods are suitable for routine monitoring.
- The analytical methods have been validated for chicken and pig tissues. The methods have not been adequately validated for cattle tissues.
- Because residue concentrations in chickens and pigs were low or non-detectable beyond 24 h withdrawal, the MRLs recommended for fat (skin/fat, where applicable) and liver are twice the LOQ of 25 μg/kg for the HPLC-UV method, and the MRLs recommended for muscle and kidney are twice the LOQ of 7 μg/kg for the HPLC-UV method. Based on the limited residue data available for cattle, it appears that residues

[1] CCα means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

are similarly low in cattle, and the recommended MRLs can be extended to cattle tissues.

The Committee recommended MRLs of 50 μg/kg for liver and fat and 15 μg/kg for muscle and kidney for chickens and pigs as narasin A. The Committee recommended the same MRLs, as temporary MRLs, for cattle.

The EDI was not estimated because there were insufficient data points to calculate the median values for residues. Using the model diet and a marker to total residue ratio of 5%, the MRLs recommended above would result in an intake of 255 μg/person per day, which represents approximately 85% of the upper bound of the ADI.

Before re-evaluation of narasin with the aim of recommending MRLs in tissues of cattle, the Committee would require a detailed description of a regulatory method, including its performance characteristics and validation data. This information is required by the end of 2010.

A residue monograph was prepared.

## 3.7 Tilmicosin

### *Explanation*

Tilmicosin is a macrolide antibiotic developed for veterinary use. It is recommended for treatment and prevention of respiratory diseases in cattle, sheep, pigs, rabbits, chickens and turkeys and for the treatment of other diseases caused by tilmicosin-sensitive microorganisms.

Tilmicosin has previously been evaluated by the Committee. The forty-seventh meeting (Annex 1, reference *125*) established an ADI of 0–40 μg/kg bw. The following MRLs were recommended for edible tissues: muscle of cattle, pigs and sheep, 100 μg/kg; fat of cattle, pigs and sheep, 100 μg/kg; liver of cattle and sheep, 1000 μg/kg; liver of pigs, 1500 μg/kg; kidney of cattle and sheep, 300 μg/kg; and kidney of pigs, 1000 μg/kg. A temporary MRL of 50 μg/l was initially recommended for milk of sheep but was not extended by the Committee at the fifty-fourth meeting (Annex 1, reference *146*), as the radiolabelled residue study in lactating sheep to determine the relationship between total residues and parent drug in milk was not available.

Tilmicosin was included in the call for data and the agenda for the current meeting of the Committee at the request of the 17th Session of CCRVDF (*7*) for evaluation of residues and recommendation of MRLs for sheep milk and poultry tissues and eggs. Data on residues in the rabbit were also submitted. This evaluation considers both new and previously submitted data.

### *Residue evaluation*

#### *Data on pharmacokinetics and metabolism*

*Ruminants.* A peer-reviewed study compared the cattle and sheep pharmacokinetics of tilmicosin after subcutaneous administration at a dose of 10 mg/kg bw. The pharmacokinetic parameters derived from the time–concentration curve were not significantly different between species.

A peer-reviewed pharmacokinetic study of tilmicosin in goats studied the bioavailability of tilmicosin after intravenous or subcutaneous administration at a dose of 10 mg/kg bw. Goat plasma and milk concentrations were determined by a microbiological assay (LOD = 5 μg/kg, LOQ = 10 μg/kg). A small fraction of the tilmicosin administered subcutaneously was absorbed very slowly. Tilmicosin was excreted in milk with a mean concentration peak of 11.6 μg/ml and depleted slowly, maintaining detectable concentrations for more than 5 days after administration.

A radiometric study was performed in cows that were approximately 2 months from calving. Radiolabelled tilmicosin was administered subcutaneously at a single dose of 10 mg/kg bw. The animals were managed as dry cows until parturition, and milk samples were collected after this time. In colostrum, tilmicosin represented 89% of the total radioactive residue, which means that the administered dose remained largely unchanged for a long period, since the interval between dosing and calving was around 50 days.

*Chickens.* Several GLP-compliant studies using $^{14}$C-labelled tilmicosin were performed. Extracts of tissue were analysed to identify metabolites that were detected using LC-MS (ESI+). Metabolites previously described in mammals were found. In liver, approximately 55% of total radioactive residue represented tilmicosin; the corresponding values for kidney and muscle were in the order of 40%.

In one study, 184 broiler chickens (92 of each sex) were treated using three concentrations of [$^{14}$C]tilmicosin in drinking-water. The averages of the administered doses were approximately 12.8, 21.8 and 56.0 mg/kg bw per day for the low dose, middle dose and high dose groups, respectively. Serial blood samples were taken from the wing veins of eight birds of each group from time 0 to 120 h after treatment. Sufficient data were available for quantitative analysis only for the high dose group. No significant differences between males and females were observed. Time to reach the maximum concentration in plasma ($T_{max}$) and other pharmacokinetic parameters could not be precisely determined because there was a data gap between 36 and 72 h and the results obtained at 72, 84 and 96 h were highly variable.

*Turkeys.* A study with unlabelled tilmicosin was performed to identify metabolites in turkey liver using LC-MS (ESI+). Parent drug was identified

as the main component, supporting tilmicosin as the marker residue for turkey.

*Eggs.* Eight laying hens received by gavage, twice a day, a dose close to 10 mg [$^{14}$C]tilmicosin/kg bw, for 3 days. Total radioactivity was determined in egg white and yolk for 24 days after the beginning of treatment. The ratio of concentrations in egg white to those in egg yolk was 1.24 ± 0.41. The median concentration in whole eggs reached a peak of 4.2 mg tilmicosin equivalents/kg on day 3. However, at this time point, the highest concentration observed was 13.7 mg tilmicosin equivalents/kg. Pools of egg whites and egg yolks were extracted and analysed by HPLC-MS/MS to identify the metabolites. A ratio of approximately 0.7 for marker to total residue concentrations was obtained.

### *Residue data*

*Dairy cows.* In a GLP-compliant study, the depletion of unlabelled tilmicosin was studied using 25 animals representing early, mid and late lactations, which received a single subcutaneous injection of 10 mg tilmicosin/kg bw. Milk samples were taken before treatment and at 2- to 3-day intervals for 42 days. The samples were analysed using an HPLC method with UV detection (LOQ = 10 μg/kg); in addition, a microbiological inhibition screening test was performed. After a 36-day withdrawal time, the concentrations were still approximately 80 μg/kg, likely to result in a positive inhibitory determination in the screening test. Tilmicosin is not registered for use in lactating cows.

*Lactating sheep.* A single subcutaneous dose of 10 mg/kg bw was given to four lactating ewes. Milk samples were taken from the animals until day 28 after the treatment. The milk was screened by a bacterial growth inhibition test and analysed for parent tilmicosin using an HPLC method with UV detection (LOQ = 50 μg/l). Full inhibition was noted for the first 6–7 days. No inhibition was found in any sample after day 12. The database was limited and did not comply with the criteria to calculate statistical parameters and tolerance limits around the depletion curve. However, based on the residue levels found, consumption of milk obtained within the first 144 h after treatment would likely result in an exceedance of the ADI, and depletion to concentrations not causing inhibitory activity would require at least a 15-day withdrawal time.

*Chickens.* One GLP-compliant depletion study using [$^{14}$C]tilmicosin was performed. After 3 days of administration, ad libitum, of [$^{14}$C]tilmicosin at a concentration in water of 75 mg/l, groups of eight animals (four of each sex) were slaughtered at 3, 7, 10, 14 and 21 days. Total radioactivity was analysed, and tilmicosin residues were determined in randomly selected samples from animals sacrificed 3 and 7 days after the end of treatment. The highest residue

concentrations were observed in liver, followed by kidney. Residue concentrations in skin fat, abdominal fat and muscle were low. The study was considered suitable for statistical evaluation and for the estimation of intakes of total residues of tilmicosin.

In another study, chickens received tilmicosin in drinking-water at an inclusion level of 75 mg/l for 3 days. Groups of 10 animals (5 of each sex) were sacrificed on days 3, 7, 10, 17 and 21 days after the end of treatment. Tilmicosin concentrations in edible tissues were determined using a validated HPLC method with UV detection, with LOQs of 60 μg/kg for liver and kidney and 25 μg/kg for muscle and fat. A statistical analysis was performed, and tolerance limits were calculated.

The data from these two studies were used to determine the ratio of marker residue to total residues.

*Turkeys.* Turkeys received tilmicosin in drinking-water at an inclusion level of 75 mg/l for 3 days. Groups of six animals (three of each sex) were sacrificed 2, 6, 10, 14 and 18 days after the end of treatment. Tilmicosin concentrations in edible tissues were determined using a validated HPLC method with UV detection, with LOQs of 60 μg/kg for liver and kidney and 25 μg/kg for muscle and fat. In liver, skin and fat, quantifiable concentrations were observed from day 2 to day 14, whereas in muscle, quantifiable results were obtained on days 2 and 6. A statistical analysis was performed, and tolerance limits were calculated. The depletion kinetics in turkeys were different from those observed in chickens.

*Rabbits.* A residue study was conducted in rabbits. The only example of a registered use of tilmicosin in rabbits made available to the Committee was for oral administration in the feed on the basis of a granulate, and the doses varied, depending on the therapeutic indication, from between 5–6 mg/kg bw and 10–12 mg/kg bw.

The animals received a single subcutaneous injection of tilmicosin calculated to result in a 10 mg/kg bw dose. Five animals (at least two of each sex) were sacrificed after various withdrawal times (7, 14, 21, 28 and 35 days), and tilmicosin concentrations were determined in liver, kidney, abdominal fat and muscle using an HPLC method with UV detection. It was validated only at concentrations far higher than those of most of the incurred residues. Thus, no valid kinetic data were obtained from this study.

*Eggs.* In a study with unlabelled tilmicosin, 15 hens (approximate age 41 weeks; body weight range of 1.59–2.15 kg) were dosed for 3 days by drinking-water. The average calculated dose was 17 mg/kg bw per day. Information on registered doses is not available, since all label copies provided by the sponsor contained a warning that tilmicosin should not be used in birds

producing eggs for human consumption. Eggs were collected from day –1 to day 23. Egg contents were analysed only for the odd days of the study. Therefore, for some animals, the highest observed concentration may not represent the peak concentration. An LC-MS/MS (ESI+) method, based on SPE with an LOQ of 25 µg/kg, was used for the analysis. The number of animals used in the study was too small to allow the derivation of MRLs. The estimated residue concentrations at day 5 were approximately 4500 µg/kg and would require long egg discard times

*Estimation of daily intake*

All intake estimates were based on the information obtained from kinetic residue depletion studies. Three approaches were followed. For residues of tilmicosin in chickens, the EDI was calculated directly from the total residue study at the same time point (7 days) on which the estimation of MRLs was based. In a second approach, a computer modelling exercise was carried out on the data to generate residue concentrations of 29 220 "food packages". This number of meals corresponds to 80 years of human life. The results showed that at 7 days' withdrawal time, the frequency of occurrence of total residues exceeding the ADI for the "food packages" in the modelling exercise was below 0.3%. The modelling also showed that for this study, the results for the median intake obtained from computer modelling and the conventionally calculated EDIs were within 0.6% of each other. The third approach was applied to estimate intakes resulting from the consumption of turkey tissues using the conventional approach involving median marker residue concentrations and factors to adjust for the ratio of marker to total residue concentrations. The factors obtained for chickens were used for turkey tissues. The estimated chronic intake calculations for chicken and turkey tissues are shown in Tables 6 and 7, respectively.

Table 6
**Estimate of chronic intake derived from total residue study in chickens on day 7**

| | Liver | Kidney | Muscle | Skin/fat | All tissues |
|---|---|---|---|---|---|
| Predicted median concentration of total residue equivalents (µg/kg) on day 7 after treatment | 2227 | 943.8 | 58.3 | 83.1 | |
| Daily amount consumed (kg) | 0.1 | 0.05 | 0.3 | 0.05 | 0.5 |
| Daily intake of total residue equivalents | 222.7 | 47.2 | 17.5 | 4.2 | 291.6 |
| % of upper bound of ADI | 9.28 | 1.96 | 0.73 | 0.18 | **12** |

Table 7
**Estimate of chronic intake derived from marker residue study in turkeys on day 7**

| | Liver | Kidney | Muscle | Skin/fat | All tissues |
|---|---|---|---|---|---|
| Predicted median concentration of marker residue concentration (µg/kg) on day 7 after treatment | 582 | 361 | 42 | 87 | |
| Daily amount consumed (kg) | 0.1 | 0.05 | 0.3 | 0.05 | 0.5 |
| Daily intake of marker residue (µg/kg) | 58.2 | 18.1 | 12.6 | 4.35 | |
| Conversion factor marker to total | 1/0.5 | 1/0.25 | 1 | 1/0.45 | |
| Daily intake of total residue equivalents (µg/kg) | 116.4 | 72.2 | 12.6 | 9.67 | 210.9 |
| % of upper bound of ADI | 4.85 | 3.01 | 0.53 | 0.4 | **8.8** |

*Analytical methods*

A validated HPLC method, based on SPE, gradient elution and UV detection, is available to analyse tilmicosin in chicken and turkey tissues. The LOQs were determined to be 60 µg/kg for liver and kidney and 25 µg/kg for muscle and fat. A validated LC-MS/MS (ESI+) method, based on SPE, is available to analyse tilmicosin in whole egg, with an LOQ of 25 µg/kg. A validated HPLC method, based on SPE, gradient elution and UV detection, is available to analyse tilmicosin in sheep milk, with an LOQ of 10 µg/l. Tilmicosin residues can be detected in milk using a commercial antimicrobial growth inhibition test.

*Maximum residue limits*

The Committee considered data for recommending MRLs in chickens, turkeys, eggs, rabbits and sheep milk. The sponsor provided information on registered uses, which showed that there is at present no registered use for laying birds. The residue concentrations in eggs were very high and could result in long withdrawal times.

In the rabbit, the residue depletion study was performed using subcutaneous administration. However, the registered oral administration route in rabbits was not covered by an adequate residue depletion study.

The argument of the sponsor that a radiolabelled residue study in sheep milk was not necessary, as new data were provided to bridge between cattle and sheep, was accepted in principle. The only residue study in lactating ewes contained an insufficient number of animals to allow MRLs to be recommended and showed that long milk withdrawal times of approximately 15 days may be required.

For chickens, a satisfactory data set was available to derive MRLs. For turkeys, the available residue data did not include a total residue study, but the data could be bridged by using the ratios of marker to total residue concentrations derived from the study in chickens.

When recommending MRLs, the Committee considered the following points:

— The ADI for tilmicosin was 0–40 μg/kg bw, corresponding to an upper bound of acceptable intakes of 2400 μg per day for a person with a body weight of 60 kg.

— The time point on which the MRLs were set was based on an EDI < ADI approach *and* on modelling of possible intakes resulting from the consumption of the four standard edible tissues showing that >99.7% of all intakes in an 80-year lifetime would be below the ADI.

— The residue depletion kinetics in turkeys were different from those found in chickens.

— The most suitable time point for the calculation of MRLs was 7 days after the end of treatment in chickens and turkeys.

— The studies provided clear evidence of dose linearity of the residues in tissues of chickens.

— The range of therapeutic doses was covered by the studies performed in chickens. The dose used in the depletion study with turkeys was at the lower end of the registered dose regimes; however, the residue data from turkeys showed less variability than did the data from chickens.

— A total residue study in chickens could be directly used for the intake estimates following adjustment to account for the slightly higher range of therapeutic doses.

— The data from the marker residue study enabled statistical MRL calculations for chickens and for turkeys. MRLs were calculated on the basis of upper one-sided 95% confidence limits over the 95th percentile of residue concentrations.

— The ratio of marker to total residue concentrations was determined for chicken tissues and applied for the estimated intake of residues from turkey tissues.

— Data submitted to support MRLs for rabbit tissues, chicken eggs and sheep milk were not suitable to derive MRLs compatible with the registered conditions of use for tilmicosin.

— A validated method of analysis was available for chicken and turkey tissues.

The Committee recommended MRLs, determined as tilmicosin, as follows:

- *For chicken*: Muscle, 150 μg/kg; liver, 2400 μg/kg; kidney, 600 μg/kg; and skin/fat, 250 μg/kg
- *For turkey*: Muscle, 100 μg/kg; liver, 1400 μg/kg; kidney, 1200 μg/kg; and skin/fat, 250 μg/kg

The Committee was not able to recommend an MRL for sheep milk.

Before a re-evaluation of tilmicosin with the aim to recommend MRLs in tissues of rabbits, the Committee would require adequately designed residue studies with doses and routes of administration under authorized conditions of use and using a validated method suitable for the purpose.

An addendum to the residue monograph was prepared.

## 3.8 Triclabendazole

### *Explanation*

Triclabendazole is a benzimidazole anthelminthic used for the control of liver fluke, *Fasciola hepatica* and *F. gigantica*, in cattle, sheep and goats. It is related by chemical structure and pharmacological action 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 0–3 μg/kg bw and recommended MRLs for cattle and sheep. At the sixty-sixth meeting of the Committee (Annex 1, reference *181*), new studies on metabolism, bioavailability, residue depletion and analytical methods were considered, and MRLs were revised and expressed as the sum of the extractable residues oxidized to ketotriclabendazole. The following MRLs were recommended for edible tissues of cattle, sheep and goats: muscle, 150 μg/kg; liver, 200 μg/kg; kidney, 100 μg/kg; and fat, 100 μg/kg.

The 17th Session of CCRVDF (*7*) requested that the MRLs for triclabendazole in cattle and sheep tissues be re-evaluated, including reconsideration of the data on bioavailability. In addition, three new residue studies in cattle using pour-on formulation were provided for evaluation by the current meeting of the Committee.

### *Residue evaluation*

#### *Data on pharmacokinetics and metabolism*

No new studies on pharmacokinetics or metabolism were provided. Re-evaluation of the data considered at the fortieth and sixty-sixth meetings of

the Committee confirmed the earlier findings. Studies investigating the bioavailability of incurred residues of triclabendazole were also reconsidered. Three studies on bioavailability were evaluated by the sixty-sixth meeting of the Committee. One study investigated the bioavailability of incurred residues in rats on the basis of blood concentrations of [$^{14}$C]triclabendazole following intravenous administration, oral administration by gavage or in feed and lyophilized [$^{14}$C]triclabendazole-derived residues from cattle tissue administered either in feed or by oral gavage. Measurements of areas under the radioactivity–time curve showed that the absolute bioavailability of [$^{14}$C]triclabendazole was approximately 70% when given by gavage in rats. The absolute bioavailability of incurred residues in tissue was the highest for liver, at 9.2%. The calculated bioavailability of incurred liver residues in cattle in this study was 13% (9.2/70 × 100) relative to the oral gavage treatment. The other two studies were in bile duct–cannulated rats. These studies confirmed that the bioavailability of incurred residues from liver was higher than that for muscle or kidney; however, the relative bioavailability could not be calculated based on the data from these studies. The relative bioavailability for liver of 13% was used in the calculation of the EDI, as it represented the worst-case scenario.

The sixty-sixth meeting of the Committee evaluated new studies on the ratios of marker residue concentration to total residue concentration in edible tissues. The available information for cattle was limited to two male animals slaughtered on day 28 after the last treatment. The ratios of marker to total residue concentrations were as follows: liver, 0.19 and 0.24; kidney, 0.24 and 0.27; and muscle, 0.41 and 0.32. For sheep, the ratios of marker to total residue concentrations were 0.25 for liver and 0.4 for muscle on day 28 and 0.42 for muscle on day 10.

### *Residue data*

The sixty-sixth meeting of the Committee considered a GLP-compliant study in which 24 cattle were dosed orally with 18 mg triclabendazole/kg bw on day 1 and again 28 days later. Highest residues measured as ketotriclabendazole 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 current meeting of the Committee considered three new GLP-compliant residue depletion studies in cattle. The pour-on formulation used in all three studies was identical. In the first study, no data from storage stability studies were provided to confirm the stability of residues under the study conditions. Two earlier studies found that triclabendazole residues in liver declined to

72% and 83% of the initial concentration, respectively, after 6 months in storage. Therefore, the data from this new residue depletion study could not be considered. The second and third studies did not generate residue depletion data on day 28, the only time point when the ratios of marker to total residue concentrations are known. Therefore, the EDI could not be calculated, and the studies were not suitable for deriving MRLs.

The sixty-sixth meeting of the Committee evaluated a GLP-compliant study in which 24 lambs received a single oral dose equivalent to 10–13 mg triclabendazole/kg bw. Six animals were slaughtered at each of the time points 14, 28, 42 and 56 days post-treatment. Two control animals did not receive the treatment and were slaughtered before the first group of treated animals was slaughtered. Samples of liver, kidney, muscle and fat were collected from each animal and analysed for residues of ketotriclabendazole by liquid chromatography with UV detection. Samples of some tissues were not analysed from the latter collection dates, as all samples of these tissues collected at the previous date had been below the LOQ. The most persistent residues were found in muscle and were detectable in three of six animals at 56 days post-treatment. Residues were not detected in liver or kidney samples at 42 days post-treatment. Quantifiable concentrations were found on days 14 and 28 in kidney and liver. In fat, all concentrations were below 50 μg/kg.

*Analytical methods*

The sixty-sixth meeting of the Committee reported that a suitable validated LC method with UV detection was available for regulatory use to detect and quantify residues as ketotriclabendazole. The demonstrated method LOQ was 0.05 mg/kg for the various tissues. Analytical recoveries ranged from 79% to 102%. Two analytical methods based on this method were provided for evaluation by the current meeting of the Committee. In all three methods, tissues are initially digested with hot alkali solution to release bound residues; after further cleanup, the extracted residues are oxidized to ketotriclabendazole, the marker residue.

*Maximum residue limits*

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

- 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.
- The marker residue is the sum of all residues extracted and converted to ketotriclabendazole.
- Liver and muscle are suitable target tissues.

— A validated analytical method is available for analysis of triclabendazole residues in edible tissues of cattle and sheep.

— The bioavailabilities of [$^{14}$C]triclabendazole and [$^{14}$C]triclabendazole-derived incurred residues administered to rats by oral gavage were 70% and 9.2%, respectively. Based on these data, the relative oral bioavailability of incurred residues was 13%.

— In cattle, the ratios of marker residue concentration to total residue concentration were 0.32 for muscle, 0.19 for liver, 0.24 for kidney and 0.4 for fat on day 28. In sheep, the ratios were 0.4 for muscle, 0.25 for liver, 0.24 for kidney and 0.4 for fat (a conservative value based on that for fat from cattle).

— The kinetic behaviour of triclabendazole is distinctly different in cattle and sheep, and there is no basis for establishing MRLs with the same numerical values for the two species.

— MRLs for liver, kidney and muscle for cattle and sheep were derived from the curve describing the upper one-sided 95% confidence limit over the 95th percentile of the residues of the marker residue ketotriclabendazole on day 28 after the last treatment and are thus higher than those recommended by the sixty-sixth meeting of the Committee, which were based on the time point of 56 days.

— MRLs for fat were based on twice the LOQ of the analytical method.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, expressed as the marker residue, ketotriclabendazole: muscle, 250 μg/kg; liver, 850 μg/kg; kidney, 400 μg/kg; and fat, 100 μg/kg. The Committee also recommended MRLs for triclabendazole for the edible tissues of sheep, expressed as the marker residue, ketotriclabendazole: muscle, 200 μg/kg; liver, 300 μg/kg; kidney, 200 μg/kg; and fat, 100 μg/kg.

The MRLs previously recommended by the sixty-sixth meeting of the Committee for triclabendazole for cattle and sheep were withdrawn. As the Committee recommended significantly different MRLs for cattle and sheep and upon reviewing the limited database for residues in goats, the Committee concluded that there were insufficient data to extend the recommended MRLs for goats. Therefore, the MRLs for goats recommended at the sixty-sixth meeting of the Committee were withdrawn.

*Estimated daily intake*

The median concentrations of the marker residue in cattle tissues were derived from the predicted value of the regression line on day 28. This was

the only time point when the ratios of marker to total residue concentrations are known. The study involved the oral treatment of 24 cattle with 18 mg triclabendazole/kg bw and is described above. The median residue concentrations on day 28 were as follows: liver, 423 μg/kg; kidney, 173 μg/kg; muscle, 161 μg/kg; and fat, 100 μg/kg (a conservative estimate in the absence of an observed value).

The residue depletion study in cattle dosed orally was evaluated statistically. The points on the curve describing the upper one-sided 95% confidence limit over the 95th percentile and the linear regression line at day 28 were derived for muscle, liver and kidney. The corresponding values for fat are, of necessity, conservative estimates, because observed values are not available. The EDI was calculated at day 28 using the median residue concentrations, the ratios of marker to total residue concentrations, food consumption values derived from the standard food basket and the bioavailability of incurred residues (13%). The EDI was calculated to be 55.5 μg/60-kg person, which represents 30.8% of the upper bound of the ADI.

The residue depletion data for sheep were analysed using the statistical evaluation approach described above. The available database for sheep was smaller than that for cattle, since measurable quantities of the marker residue were found only on days 14 and 28 in kidney and liver. The median concentrations of the marker residue in the edible tissues of sheep were calculated for day 28. With kidney, the ratio of marker to total residue concentration for cattle was used. In fat, all concentrations were less than 50 μg/kg; accordingly, a conservative value for fat of 50 μg/kg was used. The median residue concentrations on day 28 were as follows: liver, 154 μg/kg; kidney, 93 μg/kg; and muscle, 103 μg/kg. For fat, a conservative value of 50 μg/kg was used. The EDI was calculated to be 21 μg/person, which represents 11.7% of the upper bound of the ADI.

A residue monograph was prepared.

## 3.9 Tylosin

### *Explanation*

Tylosin is a macrolide antibiotic produced by fermentation from a strain of the soil microorganism, *Streptomyces fradiae.* The compound is active against Gram-positive bacteria, mycoplasma and certain Gram-negative bacteria. Like other macrolide antibiotics, tylosin inhibits protein synthesis by inhibiting aminoacyl-tRNA and peptidyl-tRNA binding to the ribosomes.

Tylosin consists of one major factor, tylosin A. Three minor factors—desmycosin (tylosin B), macrocin (tylosin C) and relomycin (tylosin D)—may also be present in varying amounts, depending upon the manufacturing source. Most of the microbiological activity resides with tylosin A. Tylosin B, C and D and dihydrodesmycosin (a metabolite) have approximately 83%, 75%, 35% and 31% of the activity of tylosin A, respectively.

Tylosin and its phosphate and tartrate salts are used in pigs, cattle and poultry for the treatment of infections caused by organisms sensitive to tylosin. They may be administered to calves, orally in the milk replacer, at a dose of 40 mg/kg bw and to cattle, by intramuscular injection, at a dose of 4–10 mg/kg bw. In pigs, tylosin is used for the prevention and control of diseases such as swine dysentery and enzootic pneumonia. It may be administered in the drinking-water at a dose of 25 mg/kg bw, in the feed at a dose of 3–7 mg/kg bw or by intramuscular injection at a dose of 2–10 mg/kg bw. Tylosin is administered to poultry in the drinking-water at a dose equivalent to 75 mg/kg bw. It is used primarily in the treatment of chronic respiratory disease complex in chickens and infectious sinusitis in turkeys caused by *Mycoplasma gallisepticum*. Tylosin has also been approved for emergency use in the control of American foulbrood of honey bees.

Tylosin was evaluated at the twelfth meeting of the Committee in 1968 (Annex 1, reference *17*). It was concluded that tylosin used in animal feed or in veterinary medicine should not give rise to detectable residues in edible products of animal origin. No ADI was established.

The drug was subsequently evaluated at the thirty-eighth meeting of the Committee in 1991 (Annex 1, reference *97*). Because of deficiencies in the toxicological and microbiological data, the Committee was not able to establish an ADI. Before reviewing the compound again, the Committee wished to have the following information:

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 is 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.

At the sixty-sixth meeting of the Committee in 2006 (Annex 1, reference *181*), none of the requested information was provided. A review of the available toxicological data in the published scientific literature indicated that publicly available data were insufficient to allow a toxicological and residue evaluation of tylosin to be performed. Therefore, tylosin could not be evaluated at the sixty-sixth meeting.

Tylosin was included on the agenda and call for data for the current meeting of the Committee as a result of a request from the 17th Session of CCRVDF (*7*).

New data on microbiological effects and detailed information on the reproductive and developmental toxicity studies reported at the thirty-eighth meeting were made available for the present meeting. This report consolidates the available information for tylosin relevant to the establishment of an ADI and MRLs.

The Committee considered the results of studies on pharmacokinetics, pharmacology, acute and short-term toxicity, genotoxicity, fertility and developmental toxicity, microbiological safety and studies in humans. The genotoxicity and microbiological studies and some acute toxicity 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 were available in summary form only and thus could not be independently assessed.

### *Toxicological and microbiological evaluation*

#### *Biochemical data*

Administration by the oral route in rats and dogs gave rise to peak serum concentrations within 2 h, followed by a rapid decline. Based on the findings in dogs, absorption appeared to take place in the intestine rather than in the stomach. In dogs given increasing doses, absorption was less than proportional to dose. Limited tissue distribution studies in rats with radiolabelled drug revealed higher levels in liver and kidney than in fat. The urinary recovery of oral doses in rats and dogs accounted for 1–2% of the dose, with the remainder in the faeces. Biliary excretion was not quantified, but tylosin

concentrations were considerably higher in bile than in serum in both rats and dogs.

The biotransformation of tylosin was extensive in rats. Major compounds found in the liver were tylosin A, relomycin and dihydrodesmycosin. In the faeces, major compounds were relomycin and dihydrodesmycosin, and minor compounds included tylosin A, macrocin and a range of metabolites arising from hydrolysis of the lactone ring.

*Toxicological data*

Tylosin was relatively non-toxic in mice, rats and dogs after single oral dosing with the base and a variety of salts. The oral $LD_{50}$ was generally >5000 mg/kg bw in rodents and >800 mg/kg bw in dogs.

Several short-term toxicity studies were performed in rats and dogs that received oral doses. Studies of up to 1 month in duration were provided in summary form only and were unsuitable for evaluation. In a 1-year rat study, there were increases in the numbers of lymphocytes and urinary pH was elevated at doses of 5000 mg/kg in the feed and above. The NOAEL in rats was 1000 mg/kg in the feed, equal to 39 mg/kg bw per day.

In a 2-year dog study, tylosin was given by capsule at doses of 0, 1, 10, 100, 200 or 400 mg/kg bw per day. Pyelonephritis was seen at 200 mg/kg bw per day, and pyelonephritis, nephrosis and cystitis were seen at 400 mg/kg bw per day. The NOAEL in dogs was 100 mg/kg bw per day.

Five long-term studies in orally dosed rats were available. Three studies were provided in summary form only and were unsuitable for evaluation. In the other studies, survival was enhanced by exposure to tylosin. In one study, fatty change in the liver was increased at a dose of 10 000 mg/kg in the feed, equivalent to 500 mg/kg bw per day. The other study revealed increased food intake at 5000 and 10 000 mg/kg in the feed and body weight gain at 10 000 mg/kg in the feed. At these same doses, the incidences of pituitary adenomas were increased in males. This type of tumour is commonly observed in ageing rats, and the apparent increase in some treated groups was considered to be associated with the increased survival in treated males. The overall NOAEL in rats was 5000 mg/kg in the feed, equal to 192 mg/kg bw per day.

Tylosin was evaluated for potential genotoxicity in an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells, in an in vitro assay for the induction of gene mutations in $HGPRT^+$ CHO cells, in an in vitro chromosomal damage assay in CHO cells and in an in vivo assay for cytogenetic damage in mouse bone marrow. Negative findings were obtained in CHO cells investigated for gene mutations and chromosomal damage and

in mouse bone marrow investigated for cytogenetic damage. An increase in gene mutations was detected in mouse lymphoma cells, in the absence of metabolic activation only. Owing to the markedly decreased survival of cells, the positive mutagenicity in the mouse lymphoma assay was considered to represent an unreliable result. It is concluded that tylosin presents a low potential for genetic damage.

Multigeneration reproduction studies were conducted in mice and rats. No adverse effects were found on reproductive indices or on growth and survival of offspring. The NOAEL was 10 000 mg/kg in the feed in all studies, equivalent to 1500 mg/kg bw per day in mice and 500 mg/kg bw per day in rats.

In developmental studies, fetal growth and development were unaffected at the highest administered doses of 1000 mg/kg bw per day in mice (given by gavage) and 725 mg/kg bw per day in rats (given as 10 000 mg/kg in the feed).

The most relevant study for determining a toxicological NOAEL is the 2-year study in dogs. The NOAEL was 100 mg/kg bw per day, and a safety factor of 100 was considered appropriate. Therefore, an ADI of 0–1 mg/kg bw could be established on the basis of the toxicological data.

### *Microbiological data*

A JECFA decision tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with VICH GL36 (*8*) was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for tylosin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were presented. A consistent absence of antibacterial activity against any of the strains of *E. coli*, which had MIC values >128 μg/ml, was apparent. Antibacterial activity was demonstrated among strains from other species. The most susceptible groups were the Gram-positive anaerobes—*Bifidobacterium*, *Clostridium*, *Eubacterium* and *Peptostreptococcus*. The $MIC_{50}$ of both *Bifidobacterium* spp. and *Clostridium* spp. was 0.062 μg/ml.

Faecal binding experiments were conducted using tylosin concentrations between 0 and 3.3 μg/ml. The reference bacterial strain was *Enterococcus faecalis*, which is susceptible to tylosin. The binding of tylosin to faeces was not influenced by the concentration of faeces present (up to 50%), but it was time dependent. Maximal binding of tylosin to faeces at each concentration occurred between 1 and 8 h, and the extent of the binding varied between 28.6% and 42.9%. Practical limitations made it impossible to perform in vitro faecal binding assays using faecal concentrations that exceeded 50% w/v. Therefore, the 50% concentration provided the closest possible in vitro representation of the in vivo situation with regard to the binding of ingested tylosin residues to intestinal contents. On this basis, it was estimated that

maximal tylosin binding of undiluted faecal material would occur within 1–8 h and would probably exceed 30%.

In studies in human volunteers given up to 20 mg tylosin orally for 6 months, there was no evidence for an increase in resistant microorganisms in the faeces or for the emergence of cross-resistance to other antibiotics.

Tylosin residues may disrupt the colonization barrier of the human gastrointestinal tract, as tylosin is most active against *Bifidobacterium* spp. and *Clostridium* spp. In terms of the development of resistance, all of the bacterial species representative of the human gastrointestinal tract had MIC values above the incurred active tylosin concentrations present in the human gastrointestinal tract. Thus, the small amount of active tylosin present in the human gastrointestinal tract would exert negligible pressure in selecting for resistance. Consequently, there is no need to consider the development of resistance as an end-point of concern.

The formula for calculating the upper bound of the microbiological ADI is as follows:

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

The equation terms are derived as described below.

*$MIC_{calc}$*: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC) ($MIC_{calc}$) for colonization barrier disruption uses MIC values from the lower 90% confidence limit (CL) of the mean $MIC_{50}$ for the most relevant and sensitive human colonic bacterial genera.

$MIC_{calc}$ is derived as follows:

$$\text{Lower 90\% CL} = \text{Mean MIC}_{50} - \frac{\text{Std Dev}}{\sqrt{n} \times t_{0.10,\text{df}}}$$

where:

- Mean $MIC_{50}$ is the mean of the log-transformed $MIC_{50}$ values;
- Std Dev is the standard deviation of the log-transformed $MIC_{50}$ values;
- $n$ is the number of $MIC_{50}$ values used in the calculations; and
- $t_{0.10,df}$ is the 90th percentile from a central $t$-distribution with degrees of freedom (df) = $n - 1$.

The tylosin ADI was derived from in vitro MIC data as described in VICH GL36 (*8*). The strains needed to determine the $MIC_{calc}$ were chosen according

to these guidelines, which state that an intrinsically resistant bacterial genus should not be included; thus, *E. coli* was excluded. Additionally, although VICH GL36 requires the use of genera with a $MIC_{50}$, the Committee used the same genera but used the $MIC_{90}$ to determine the $MIC_{calc}$. The justification is based on Clinical and Laboratory Standards Institute (CLSI) guidelines (*16*) and the observation that some of the strains within each of the genera tested exhibited high MICs, presumably due to resistance. Given that the strains with the high MICs are more likely to fill niches vacated by susceptible strains (i.e. those with low MICs), the argument is advanced that these strains represent a more realistic scenario of what might occur in the human colon.

The $MIC_{90}$ values for *Bacteroides fragilis*, other *Bacteroides* spp., *Bifidobacterium* spp., *Clostridium* spp., *Enterococcus* spp., *Eubacterium* spp., *Fusobacterium* spp., *Lactobacillus* spp. and *Peptostreptococcus* were used to obtain the following values:

- $MIC_{90} = 5.44$
- $t = 1.397$
- Lower 90% CL = 3.76
- $MIC_{calc} = 1.698$

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

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

The mix of metabolites reaching the colon would have 35% of the activity of tylosin A based on results in pigs as a surrogate model for the human. The faecal inactivation study indicates that at least 36% of tylosin A is bound to faeces, so 64% is available to microorganisms. Multiplying these two "availability" factors together (i.e. $0.35 \times 0.64$) gives 0.224. This is the fraction of an oral dose available to microorganisms.

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

Therefore, the upper bound of the ADI is calculated as follows:

$$\text{Upper bound of ADI} = \frac{1.698 \times 200 \text{ g}}{0.224 \times 60 \text{ kg}}$$

$$= 25.3 \text{ µg/kg bw}$$

Therefore, a microbiological ADI of 0–30 µg/kg bw (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing and faecal binding data.

*Evaluation*

The sponsor addressed the concerns expressed by the Committee at its thirty-eighth meeting, which enabled a reconsideration of all the toxicological and related information and a review of new studies on microbiological effects. The Committee considered that microbiological effects were more relevant than toxicological effects for the establishment of an ADI for tylosin. Therefore, the Committee established an ADI of 0–30 µg/kg bw on the basis of the MIC data. This ADI is significantly lower than it would be if it were based on a toxicological end-point and is 6400-fold lower than the lowest dose associated with increased pituitary tumours in rats.

A toxicological monograph was prepared.

## *Residue evaluation*

*Data on pharmacokinetics and metabolism*

Tylosin is a highly lipid soluble weak organic base ($pK_a = 7.73$) that readily forms salts and esters. It is slightly to moderately bound to plasma proteins (30–47%) and is widely distributed in body fluids and tissues. The volume of distribution ($V_d$) of tylosin ranges from 1 to 14.6 l/kg in various animal species.

Following intravenous administration, tylosin is rapidly eliminated from blood, with elimination half-lives ranging from 0.4 to 4.5 h in several animal species. Total body clearance of tylosin was highest in rodents (>70 ml/min per kilogram) and lower in adult cattle, sheep and goats (<10 ml/min per kilogram).

The biotransformation of tylosin has been studied in rats, chickens, pigs and cattle, and the comparative metabolism was shown to be qualitatively similar for these species. Tylosin is principally metabolized in the liver, resulting in four major metabolites and several minor metabolites in most of the species studied. Tylosin A is the most abundant residue in cattle, pigs, chickens and

rats, whereas a major metabolic pathway is the reduction of tylosin A to tylosin D.

*Residue data*

Two calves weighing approximately 150 kg each were treated once daily for 3 days with intramuscular injections of $^{14}$C-labelled tylosin at a dose of 17.6 mg/kg bw. Four hours after the last dose, the calves were slaughtered and tissues were taken for analysis. The mean total residues of tylosin (expressed as milligrams tylosin equivalents per kilogram) were 25.2 (liver), 47.8 (kidney), 2.9 (muscle) and 1.5 (fat). In liver, tylosin A was the main component of the residue, whereas tylosin D, tylosin C and cysteinyl-tylosin A were major metabolites. The residues in kidney and fat demonstrated similar metabolic profiles, with tylosin A and cysteinyl-tylosin A being major components. By comparison, tylosin A was the only significant residue in muscle. The mean residues of tylosin A analysed by HPLC were 2.6 mg/kg (liver), 6.9 mg/kg (kidney), 0.7 mg/kg (muscle) and 0.9 mg/kg (fat), corresponding to 10.5%, 14.5%, 24.6% and 61.8% of the total residues in these tissues. Tylosin A represented 31% (liver), 36.7% (kidney) and 70% (muscle) of the microbiologically active residues present.

Using a crossover study design with a 21-day washout period, tylosin was administered intramuscularly and subcutaneously to 12 cattle at a dose of 17.6 mg/kg bw for 5 consecutive days. The animals were slaughtered 21 days after the last treatment, and samples of liver and kidney were collected for residue analysis. No residues of tylosin A in liver or kidney were detected (LOD of 20 μg/kg) by HPLC with UV detection.

Three crossbred castrated male pigs weighing approximately 17 kg were given $^{14}$C-labelled tylosin in feed at an inclusion rate of 220 mg/kg for 5 days. The animals were slaughtered 4 h after the last dose, and tissues were collected for residue analysis. The mean total residues of tylosin, expressed in micrograms tylosin equivalents per kilogram, were 450 (liver), 460 (kidney), 70 (muscle), 50 (fat), 170 (lung) and 70 (skin). Residues of tylosin A were not detected when these samples were analysed by HPLC with UV detection; the method LOQ was 50 μg/kg in all tissues. Tylosin A accounted for 12.3% of the total residue in liver and 7.6% in kidney. Smaller amounts of tylosin D (liver, 10.3%; kidney, 6.1%), dihydrodesmycosin (liver, 5.4%; kidney, 4.1%) and cysteinyl-tylosin A, which readily converts to tylosin A, were also present.

From other residue studies using injectable formulations, residues at the injection sites were noted in cattle and pigs. In cattle, the mean concentration of tylosin at the injection site was 1621 μg/kg at 7 days and less than the LOD from day 28 after treatment. In pigs, the mean concentration of tylosin at the

injection site was 1040 µg/kg at day 3 and less than the LOD from day 10 after treatment. Injection site residues were noted but were not taken into account when recommending the MRL for muscle for cattle and pigs.

A radiometric study was conducted in which laying hens had ad libitum access to drinking-water medicated with 0.53 g [$^{14}$C]tylosin/l for 3 days. Samples of liver, kidney, muscle, skin with adhering fat and abdominal fat were collected from each of four animals sacrificed at 0, 2, 5 and 7 days after withdrawal of the medicated water. Total radioactive residues in liver for the four birds at 0-day withdrawal (4 h) were 13.7, 1.0, 0.5 and 0.5 mg [$^{14}$C]tylosin equivalents/kg. The mean total radioactive residue in liver declined to less than 0.1 mg tylosin equivalents/kg by 7 days after withdrawal. By comparison, the mean total residue in kidney decreased to below 0.1 mg tylosin equivalents/kg by 2 days after withdrawal; the mean total residue in skin with adhering fat and in abdominal fat decreased to below 0.1 mg tylosin equivalents/kg at all sampling times. Tylosin A was the principal component of the residue in liver, accounting for approximately 16% of the total residue.

In another study, broiler chickens were given 0.53 g [$^{14}$C]tylosin/l of drinking-water for 3 days. Samples of liver, kidney, muscle, skin with adhering fat, abdominal fat and bile were collected from six animals at 0, 2, 5 and 7 days after withdrawal of the medicated water. The mean total radioactive residue in liver declined from 0.7 mg tylosin equivalents/kg at day 0 to less than 0.1 mg tylosin equivalents/kg by 5 days after withdrawal. In kidney, the mean total radioactive residue decreased to less than 100 µg tylosin equivalents/kg by 5 days after withdrawal. Mean total radioactive residues in muscle, skin and abdominal fat were less than 100 µg tylosin equivalents/kg at all time points. The liver extract contained multiple radioactive components, indicating extensive metabolism; however, tylosin D was the only residue detected by HPLC-MS/MS (ESI) on account of the low residue concentrations and reduced assay sensitivity due to matrix effects.

The depletion of tylosin residues in muscle, liver, kidney and skin/fat of broiler chickens was studied following oral administration of tylosin in drinking-water at a rate of 500 mg/l for 5 days. This is equivalent to a mean daily dose of tylosin of approximately 105 mg/kg bw. Groups of chickens (three males and three females per group) were euthanized at 0, 12, 24 and 48 h after withdrawal of the medicated water, and samples of liver, kidney, muscle and skin/fat were collected and analysed for tylosin A (marker residue) using a validated HPLC-MS/MS method. The method LOQ was 50 µg/kg for all tissues. Residues of tylosin in liver, kidney, muscle and skin/fat were less than 100 µg/kg at 0 h and approached, or were less than, 5 µg/kg (the LOD of the method) at 12 and 24 h after the medicated water had been withdrawn.

Twelve cows were given tylosin phosphate in feed for 17 days, equivalent to a dose rate of 200 mg/cow per day. Milk samples were collected and assayed for tylosin on days –1, 0 (first access of the animals to medicated feed), 1, 2, 3, 4, 5, 7 and 17 by HPLC with UV detection. Tylosin residues were not quantifiable (LOQ of 50 μg/kg) in any milk sample.

Five high-yielding and five low-yielding dairy cows were treated intramuscularly for 5 days with 10 mg tylosin/kg bw. Milk samples were analysed for tylosin using an HPLC method with UV detection. The method LOQ and LOD were 25 μg/kg and 10 μg/kg, respectively. The maximum concentration in milk ranged from 1.3 to 2.6 mg/kg on the 4th day of treatment. The concentration of tylosin residues in all samples was less than 50 μg/kg from day 3 after the last dose. There was no conclusive evidence that the marker residue was correctly quantified in this study.

Six dairy cows were administered 10 mg tylosin/kg bw intramuscularly for 3 days. Milk was collected twice daily from 1 day prior to treatment to 5 days after the last treatment and analysed for tylosin using a validated HPLC method with UV detection. The LOQ and LOD of the analytical method were 50 μg/kg and 20 μg/kg of milk, respectively. The highest concentrations of tylosin A residues were observed in milk during treatment, with mean tylosin A concentrations of 1.1 mg/kg, 1.5 mg/kg and 1.4 mg/kg on days 1, 2 and 3, respectively. Concentrations of tylosin A were less than the LOQ (<50 μg/kg) at the afternoon milking on day 3 post-treatment and less than the LOD (<20 μg/kg) at the morning milking on day 4 post-treatment.

Laying hens were given ad libitum access to drinking-water medicated with 0.53 g [$^{14}$C]tylosin/l for 3 days. Eggs were collected daily from all birds during the treatment period, after withdrawal of the medicated drinking-water and prior to sacrifice. Total residues present in whole eggs at 0-day withdrawal from 2 of the 16 treated birds were 1.6–1.7 mg tylosin equivalents/kg of eggs, whereas the residues present in whole eggs from the remaining 14 birds ranged from 108 to 245 μg equivalents/kg. This difference was not ascribed to any clinical or physiological observation. Residue concentrations in whole eggs were highest in eggs collected on the last day of treatment (mean of 333 μg tylosin equivalents/kg) and on the following day (mean of 362 μg tylosin equivalents/kg). Thereafter, mean residues in whole eggs depleted to 190, 134, 126 and 70 μg tylosin equivalents/kg at 1, 2, 3 and 4 days after withdrawal of the medicated water, respectively. Tylosin A was the most abundant of the identified residues in whole eggs. At the highest concentration of tylosin equivalents, the mean tylosin A concentration was 272 μg/kg and accounted for approximately 17% of the total radioactive residues. Tylosin A was not detected in eggs produced by the other 14 birds (the LOD of the analytical method was approximately 20 μg/kg).

The residue depletion profiles of tylosin in eggs were investigated in 24 laying hens after oral administration of tylosin phosphate in the diet. The birds received tylosin phosphate at an inclusion rate of 800 mg/kg in feed for 5 consecutive days. Tylosin residue levels were determined in eggs produced from the day before dosing to 5 days after the suspension of dosing. The HPLC method with UV detection was not specific for tylosin A; the LOQ was 50 μg/kg, and the LOD was 13 μg/kg for tylosin residues. One egg collected on the 5th day of dosing contained a residue of 75 μg tylosin/kg. The concentration of residues in all other eggs was less than the method LOQ.

In another study, 17 hens were offered water medicated with 500 mg tylosin activity/l for 3 days. The residues of tylosin in 12 eggs randomly selected each day were determined by HPLC with UV detection, which was not specific for tylosin A. Only 4 of 36 eggs collected during the treatment period contained residues greater than the LOQ (50 μg tylosin/kg) of the analytical method. After withdrawal of the medicated water, no residue concentrations exceeded the LOQ, with the majority of eggs having residues below the LOD (10 μg tylosin/kg).

Drinking-water medicated with 500 mg tylosin/l was given to 22 laying hens for 5 consecutive days. This was equivalent to an estimated daily dose range of 87–97 mg tylosin/kg bw. Eggs were collected daily and individually homogenized prior to analysis for tylosin A (marker residue) using a validated HPLC method. The mean concentration of tylosin in whole eggs was less than the LOQ of 50 μg/kg on all days. The maximum concentration of tylosin was 117 μg/kg in an egg from day 2 after initiation of treatment; the concentration of tylosin was less than the LOQ after day 6.

As distinct from mammalian tissues, tylosin B is a major end product in honey. The microbiological activity of both tylosin A and tylosin B must be taken into account when considering food safety. Residue depletion studies in honey were provided. A suitably validated microbiological assay was not provided to allow for the total microbiological activity of residues. Therefore, the Committee was unable to evaluate the residue data for honey.

### *Analytical methods*

An HPLC method with UV detection at 280 nm was provided for the detection and quantification of residues of tylosin A in eggs. It was claimed that the method LOQ was 50 μg/kg for whole eggs; however, a critical analysis of information provided suggested that the LOQ is likely to be above 100 μg/kg. Similarly, the LOD was claimed to be 4.1 μg/kg for whole eggs; however, on the basis of the information provided, the LOD is likely to approximate 50 μg/kg. Analytical recoveries ranged from 74.0% to 87.4%, with coefficients of variation of 3.6–9.6%. The method is not acceptable for measuring

tylosin residues at or below a concentration of 100 μg/kg, but is acceptable for measuring higher concentrations.

A validated HPLC-MS/MS method with ESI is available for determining residues of tylosin A in the edible tissues of chickens and in eggs. Acceptable specificity, sensitivity, linearity, precision, recovery and accuracy were demonstrated for the method. Recoveries ranged from 84.6% to 103%, with coefficients of variation of 5.0–10.1%. The method LOQ was 50 μg/kg for all tissues and 100 μg/kg for eggs. The LOD of the analytical method was 5 μg/kg for all tissues and eggs. The ion chromatograms and other information provided confirmed the claimed performance characteristics. There were no significant matrix effects detected in any matrices. This method could easily be extended to other matrices and is a suitable analytical method for regulatory use of residues in the edible tissues of cattle, pigs, chickens, milk and eggs.

A microbiological assay was provided for analysis of edible tissues of cattle and pigs; however, the method was not appropriately validated.

*Maximum residue limits*

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

- An ADI of 0–30 μg/kg bw based on a microbiological end-point was established by the current meeting of the Committee, equivalent to 0–1800 μg for a 60-kg person.
- The marker residue is tylosin A and represents approximately 100% of the microbiologically active residues, except in honey. This information is incorporated in the calculation of the intake estimates to ensure that they correctly reflect residues of microbiological concern.
- Liver and muscle are suitable target tissues.
- A validated analytical method is available for analysis of tylosin A residues in edible tissues of chickens and in eggs and could be extended to the edible tissues of cattle and pigs and to milk.
- The MRLs for all edible tissues of cattle, pigs and chickens were based on the data provided.
- The MRL for eggs was based on the highest tylosin A concentration observed.
- The MRL for milk was based on 2 × LOQ.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, pigs and chickens, expressed as

the marker residue, tylosin A: muscle, 100 μg/kg; liver, 100 μg/kg; kidney, 100 μg/kg; fat, 100 μg/kg (cattle and pigs); and skin/fat, 100 μg/kg (chickens). The Committee also recommended a MRL for milk of 100 μg/kg and a MRL for eggs of 300 μg/kg, both expressed as the marker residue, tylosin A.

The EDI was not estimated, because there were insufficient data points with which to calculate the median values for residues. Using the model diet and the microbiological activity of tylosin A of 100% of the microbiological activity of the residue, the MRLs above would result in an intake of 230 μg, which represents 13% of the upper bound of the ADI.

A residue monograph was prepared.

# 4. Future work

1. The Committee recommended, in the interest of transparency and communication, that the JECFA Secretariat share the concept paper on the hypothesis-driven decision tree approach for the safety evaluation of residues of veterinary drugs with relevant FAO/WHO bodies, including joint expert committees and Codex committees, while clearly communicating that this paper is a first step in a long-term project. The Committee further recommended that the JECFA Secretariat convene one or more working groups as appropriate to expand and continue the development of a general decision tree approach for the evaluation of veterinary drugs. Progress in the development of this decision tree approach should be discussed at future JECFA meetings.

2. The Committee decided to harmonize with the sixty-eighth JECFA and agreed to differentiate between the terms NOAEL and NOEL for the evaluation of veterinary drugs in food in the current and future JECFA reports.

3. The Committee recommended that the JECFA Secretariat advise the JMPR Secretariat of the Committee's report regarding residues in honey and considerations of residues from use of pesticides in honey production and bee health.

# 5. Recommendations

1. Recommendations relating to specific veterinary drugs, including ADIs and proposed MRLs, are given in section 3 and Annex 2.

2. In considering the matters of interest noted in this report concerning residues of veterinary drugs in honey and possible approaches to derive MRLs for this commodity and the complex and unique nature of honey and honey bees, JECFA may not be able to adopt any specific approaches without further guidance from CCRVDF. The Committee therefore makes the following recommendations to CCRVDF:

— That CCRVDF, with the aid of member countries, compile a comprehensive list of all veterinary drugs registered for honey production and bee health and develop a priority list of veterinary drugs for use in honey bees to be considered for risk assessment by JECFA.

— That CCRVDF and member countries be encouraged to provide data on honey consumption, considering both direct and indirect honey intake, for purposes of improved intake assessments as part of the risk assessment for recommending MRLs.

— That CCRVDF consider extension of good veterinary practice guidelines to include honey production.

— That the CCRVDF ad hoc Working Group on Methods of Analysis and Sampling consider analytical methods for residues in honey.

— That CCRVDF provide guidance on the appropriate percentile for an estimation of acute intake.

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Annex 1

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

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Annex 2

# Recommendations on compounds on the agenda and further information required

## **Avilamycin** (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–2 mg/kg bw on the basis of a NOAEL of 150 mg avilamycin activity/kg bw per day and a safety factor of 100 and rounding to one significant figure.

Residue definition: Dichloroisoeverninic acid (DIA)

**Recommended maximum residue limits (MRLs)**

| Species | Skin/fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Pigs | 200 | 200 | 300 | 200 |
| Chickens | 200 | 200 | 300 | 200 |
| Turkeys | 200 | 200 | 300 | 200 |
| Rabbits | 200 | 200 | 300 | 200 |

## **Dexamethasone** (glucocorticosteroid)

Acceptable daily intake: The Committee established an ADI of 0–0.015 µg/kg bw at the 42nd meeting of the Committee (WHO TRS No. 851, 1995).

Residue definition: Dexamethasone

**Recommended maximum residue limits (MRLs)**

| Species | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) | Milk (µg/l) |
|---|---|---|---|---|
| Cattle | 1.0 | 2.0 | 1.0 | 0.3 |
| Pigs | 1.0 | 2.0 | 1.0 | |
| Horses | 1.0 | 2.0 | 1.0 | |

### **Malachite green** (antimicrobial agent and contaminant)

Acceptable daily intake: The Committee considered it inappropriate to establish an ADI for malachite green and did not support the use of malachite green for food-producing animals.

Residues: The Committee did not recommend MRLs for malachite green and leucomalachite green, as it did not support the use of malachite green for food-producing animals. Chickens

### **Melengestrol acetate** (production aid)

Acceptable daily intake: The Committee established an ADI of 0–0.03 µg/kg bw at its 54th meeting (WHO TRS No. 900, 2001). It did not consider it necessary to reconsider the ADI at the current meeting on the basis of new data provided.

Residues: The MRLs that were recommended by the 66th meeting of the Committee (WHO TRS No. 939, 2006) were not reconsidered and were maintained.

### **Monensin** (antimicrobial agent and production aid)

Acceptable daily intake: The Committee established an ADI of 0–10 µg/kg bw on the basis of a NOAEL of 1.14 mg/kg bw per day and a safety factor of 100 and rounding to one significant figure.

Residue definition: Monensin

**Recommended maximum residue limits (MRLs)**

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) | Milk (µg/kg) |
|---|---|---|---|---|---|
| Cattle | 100 | 10 | 20 | 10 | 2 |
| Sheep | 100 | 10 | 20 | 10 | |
| Goats | 100 | 10 | 20 | 10 | |
| Chickens | 100 | 10 | 10 | 10 | |
| Turkeys | 100 | 10 | 10 | 10 | |
| Quail | 100 | 10 | 10 | 10 | |

## **Narasin** (antimicrobial agent and production aid)

| | |
|---|---|
| Acceptable daily intake: | The Committee established an ADI of 0–5 µg/kg bw on the basis of a NOAEL of 0.5 mg/kg bw per day and a safety factor of 100. |
| Residues: | Narasin A |

**Recommended maximum residue limits (MRLs)**

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Cattle | 50[a] | 15[a] | 50[a] | 15[a] |
| Chickens | 50 | 15 | 50 | 15 |
| Pigs | 50 | 15 | 50 | 15 |

[a] The MRL is temporary.

Before a re-evaluation of narasin with the aim of recommending MRLs in tissues of cattle, the Committee would require a detailed description of a regulatory method, including its performance characteristics and validation data. This information is required by the end of 2010.

## **Tilmicosin** (antimicrobial agent)

| | |
|---|---|
| Acceptable daily intake: | The Committee established an ADI of 0–40 µg/kg bw at its 47th meeting (WHO TRS No. 876, 1998). |
| Residue definition: | Tilmicosin |

**Recommended maximum residue limits (MRLs)**

| Species | Skin/fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Chickens | 250 | 600 | 2400 | 150 |
| Turkeys | 250 | 1200 | 1400 | 100 |

The Committee was not able to recommend a MRL for sheep milk.

Before a re-evaluation of tilmicosin with the aim of recommending MRLs in tissues of rabbits, the Committee would require adequately designed residue studies with doses and routes of administration under authorized conditions of use and using a validated method suitable for the purpose.

## **Triclabendazole** (anthelminthic)

Acceptable daily intake: The Committee established an ADI of 0–3 µg/kg bw at its 40th meeting (WHO TRS No. 832, 1993).

Residue definition: Ketotriclabendazole

**Recommended maximum residue limits (MRLs)**

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Cattle | 100 | 400 | 850 | 250 |
| Sheep | 100 | 200 | 300 | 200 |

## **Tylosin** (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–30 µg/kg bw based on a microbiological end-point derived from in vitro MIC susceptibility testing and faecal binding data ($MIC_{calc}$ = 1.698).

Residue definition: Tylosin A

**Recommended maximum residue limits (MRLs)**

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) | Skin/fat (µg/kg) | Milk (µg/kg) | Eggs (µg/kg) |
|---|---|---|---|---|---|---|---|
| Cattle | 100 | 100 | 100 | 100 | | 100 | |
| Pigs | 100 | 100 | 100 | 100 | | | |
| Chickens | | 100 | 100 | 100 | 100 | | 300 |