EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives

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Joint FAO/WHO Expert Committee on Food Additives
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Monographs containing summaries of relevant data and toxicological evaluations are available from WHO under the title:


Residues monographs are issued separately by FAO under the title:


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**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 International Programme on Chemical Safety (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.
1. **Introduction**

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at WHO Headquarters, Geneva, from 15 to 24 February 2000. The meeting was opened by Dr T. Meredith, Coordinator, Chemical Safety, Department of Protection of the Human Environment, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization.

Dr Meredith noted that many scientific principles and evaluation procedures had been developed by the Committee over the past 13 years during its meetings on residues of veterinary drugs in food. The Conference on International Food Trade Beyond the Year 2000, held in Melbourne, Australia, in October 1999 (1), had recommended that WHO update the scientific principles used by the Committee and the Joint FAO/WHO Meeting on Pesticide Residues to evaluate residues of veterinary drugs and other chemicals in food (food additives, pesticides and contaminants) and publish the principles in a single document. Dr Meredith reported that WHO was considering ways of responding to that recommendation. The FAO Secretariat had already consolidated the principles for the evaluation of residues of veterinary drugs in food, and the document that had been produced should be useful both to the Committee and to others interested in understanding its evaluation procedures. The document would also be useful to WHO when it critically reviews the principles for evaluating veterinary drug residues in food and consolidates them in response to the recommendation of the conference held in Melbourne.

Twelve previous 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 and 140) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984 (2). The present meeting was convened in response to a recommendation made at the fifty-second meeting of the Committee that meetings on this subject should be held annually (Annex 1, reference 140). The Committee’s purpose was 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.

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1 As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been 53 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
of animal origin. The specific tasks before the Committee were:

— 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); and
— to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2).

2. **General considerations**

2.1 **Modification of the agenda**

Temesfos was removed from the agenda since no data had been submitted.

2.2 **Interpretation of data on inhibition of cholinesterase activity**

The Committee considered the issue of interpreting data on inhibition of the activity of various cholinesterases, in particular brain and erythrocyte acetylcholinesterase and plasma and brain butyrylcholine esterase.

The Committee considered the report of a consultation on interpretation of inhibition of acetylcholinesterase activity (3) and the report of the 1998 Joint FAO/WHO Meeting on Pesticide Residues (4), which included a section on this topic. These reports were considered to have been helpful, and the Committee agreed with their conclusions. It particularly welcomed the guidance relating to methodological issues in assessing cholinesterase activity provided in Annex 1 of the report of the consultation.

The Committee agreed that inhibition of brain acetylcholinesterase activity and clinical signs of neurobehavioural effects are the endpoints of greatest concern in toxicological studies of compounds that inhibit acetylcholinesterase. The possibility of inhibition of acetylcholinesterase activity in the peripheral nervous system is also of concern, but the Committee recognized that information suitable for assessing such activity is not often available. It agreed that erythrocyte acetylcholinesterase activity could serve as a surrogate for acetylcholinesterase activity in the peripheral nervous system and brain when information on the latter was not available.

The Committee agreed that statistically significant inhibition of acetylcholinesterase activity by 20% or more should be regarded as a treatment-related effect and could form the basis for establishing an
ADI if it was the most sensitive end-point, although each compound should be considered on a case-by-case basis.

The Committee concluded that inhibition of brain and plasma butyr-ylcholinesterase activity is not of toxicological significance for establishing an ADI, but that information on inhibition of these enzymes should nevertheless be provided, as it is a useful indicator of absorption of a cholinesterase inhibitor and of occupational exposure.

2.3 Consideration of recommendations arising from an informal meeting on harmonization with the Joint FAO/WHO Meeting on Pesticide Residues

A meeting to harmonize the work of the Committee and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) was held in Rome in February 1999, at which issues relating to the evaluation of chemicals used as both pesticides and veterinary drugs were discussed. It was noted that differences in the evaluation procedures used by the Committee and JMPR had led to different approaches to the definition of residues, estimation of dietary intake, description of commodities for analysis and recommendations for MRLs. Other topics discussed at the meeting included tissue matrices used for the analysis of residues in meat/muscle, fat, milk and eggs, and risk assessment.

At its present meeting, the Committee considered the recommendations summarized in the report of the informal meeting on harmonization (5) and addressed those directed to it, which are listed below with comments.

Tissue

Clarification of the definition of muscle tissue (in Volume 3 of Residues of veterinary drugs in foods (6)) is needed to establish the portion of the commodity to which the MRL applies. Muscle tissue shall include interstitial fat and exclude trimmable fat.

The Committee agreed with the recommendation to clarify the portion of the commodity to which the MRL applies and will recommend a revised definition.

For the determination of fat-soluble pesticide/veterinary drug residues in meat/muscle for enforcement or monitoring purposes, laboratories are advised to collect and to analyse trimmable fat and to report the residues on a lipid basis, i.e. meat (fat) for JMPR and fat for JECFA. For meat without trimmable fat, the entire commodity should be analysed as meat/muscle, but only where the MRL has been set on a meat/muscle basis.

The Committee agreed that guidance for laboratories based on the proposed definitions of muscle and fat that were drafted at its present
meeting (see Annex 3) should be submitted to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration. It did, however, note that such guidance is, in general, the responsibility of national authorities.

For the determination of non-fat-soluble pesticides/veterinary drug residues in meat/muscle, laboratories are advised to analyse meat/muscle with trimmable fat removed, as far as is practical.

The Committee agreed that laboratories should analyse samples on the basis of the proposed draft definitions of muscle and fat to be submitted to the Codex Committee on Residues of Veterinary Drugs in Foods. It did, however, note that such guidance is, in general, the responsibility of national authorities.

Where JECFA and JMPR have recommended MRLs for the same chemical with the same residue/marker definition for the same commodity, the higher MRL shall prevail.

The Committee agreed that when two different MRLs exist, the higher value should prevail, provided that it does not result in the intake of residues exceeding the ADI. The risk assessment procedures used by the Committee and JMPR are designed in such a way that no adverse effects on public health would be expected to occur when residues do not exceed the MRL.

*Milk*

For the determination of fat-soluble pesticide/veterinary drug residues in milk, the milk fat portion of fresh milk should be analysed, and the results expressed on a whole milk basis using 4% as the nominal fat content.

The Committee agreed with this recommendation.

For the determination of non-fat-soluble pesticide/veterinary drug residues in milk, laboratories should analyse the whole milk and should report residues on a whole milk basis.

The Committee agreed with this recommendation.

JECFA should consider expressing MRLs for milk on a weight (kg) basis rather than the current volume (l) basis.

The Committee noted that this recommendation had already been implemented. The reporting of residue concentrations on a weight-per-weight basis is consistent with internationally recommended analytical protocols.

*Eggs*

JECFA should specify that the portion of the raw commodity "egg" (in shell) to be analysed is the whole egg white and yolk combined after removal of the shell.
The Committee agreed with the recommendation that the current definition should be changed to avoid any ambiguity about the portion of egg to be analysed for residues.

**Harmonization**

The working group noted the disparate residue definitions by the Codex Committee on Pesticide Residues and the Codex Committee on Residues of Veterinary Drugs in Foods for abamectin and recommended that the Codex Committee on Residues of Veterinary Drugs in Foods and JECFA consider expansion of its residue definition to include other isomers, such as the photodegradation isomer of B15.

The Committee carefully considered the toxicological and chemical assessments of abamectin made by JMPR and concluded that inclusion of the photodegradation isomer in the residue definition would not be consistent with the assessment of abamectin as a veterinary drug. Inclusion of other residues of abamectin would be reviewed at a future meeting of the Committee.

Cypermethrin and α-cypermethrin should remain as the marker residue definitions for their use as veterinary drugs for cypermethrin and α-cypermethrin, respectively, and cypermethrin (sum of isomers) should remain as the residue definition for the pesticide cypermethrin. Guidance should be supplied to laboratories on the designation of the measured residue as cypermethrin or α-cypermethrin based on the chromatography of the test substance.

The Committee agreed with the recommendation on definition of marker residues for the various uses. The Committee recognized that the currently available analytical methods allow measurement of all isomers of interest. Accordingly, guidance should be provided to assist Member States in providing specific advice to their residue control authorities.

Harmonization efforts should be undertaken on a case-by-case basis where marker residue definition/residue definition differences occur between JECFA and JMPR.

The Committee agreed with this recommendation and will coordinate with the JMPR Secretariat when necessary.

JECFA should review the apparent anomaly of MRLs for both fat and muscle for the fat-soluble drugs α-cypermethrin and cypermethrin. JECFA should consider which sample tissues are to be analysed by the enforcement laboratory.

The Committee agreed in principle with this recommendation, but noted that the temporary MRLs for α-cypermethrin and cypermethrin were not extended at the present meeting because the required data were not provided. The Committee indicated that some MRLs are recommended only for guidance; an example is the MRL for metrifonate in the edible tissues of cattle treated in accordance
with good practice in the use of veterinary drugs, where there are no measurable residues. Such “guidance MRLs” should not be considered in calculations of the theoretical maximum intake of residues and are not intended for residue control purposes.

For compounds that are common to both, JMPR and JECFA should use the more specific animal commodity descriptions to enhance harmonization.

The Committee agreed with this recommendation and has routinely noted the descriptions of specific animal commodities in recommending MRLs for residues of veterinary drugs in food. These descriptions are useful to JMPR in their assessment of risks associated with exposure to residues of pesticides.

Each expert panel needs a better understanding of the other’s procedures for food safety assessments for estimating MRLs and dietary exposure. . . . JECFA will provide JMPR its guidance document describing the JECFA evaluation procedures when the draft version is finalized.

The Committee agreed with this recommendation and has provided a guidance document (7) to the JMPR Secretariat. The document would also be made available to delegates at the Twelfth Session of the Codex Committee on Residues of Veterinary Drugs in Foods, to be held in March 2000.

The JECFA/JMPR group acknowledged the very different approaches used for dietary exposure determinations. JMPR will provide JECFA with detailed reports of its assessments, dietary intake calculations and per cent ADI determinations for compounds of interest to JECFA. When the data are available, JECFA will provide JMPR with median and upper limit animal commodity residue values and dietary intake calculations per cent ADI determinations for compounds of interest to JMPR.

The Committee acknowledged that the way in which it calculates dietary intake differs widely from that of JMPR. To facilitate intake assessments by JMPR, the Committee would provide data on residues of veterinary drugs of interest when they are available.

JECFA and JMPR should consider the exchange of one panel member each for a portion of the expert panel meetings to facilitate the harmonization of MRLs and risk assessment for substances used as veterinary drugs and pesticides.

The Committee agreed with this recommendation. Two JMPR members participated in the present meeting.

The FAO Joint Secretary for JMPR will attend the JECFA meeting and the FAO Joint Secretary for JECFA will attend the JMPR meeting, particularly when MRLs and risk assessments of substances used as both veterinary drugs and pesticides are being considered.

The Committee agreed that both FAO Joint Secretaries should participate in meetings at which a substance used as both a veterinary drug and a pesticide is being considered.
Joint meetings of JMPR and JECFA should be held on an ad hoc basis to address issues of mutual interest (for example, how to address MRL and ADI issues for classes of compounds with common modes of action).

The Committee agreed with this recommendation. Ad hoc meetings of members of JECFA and JMPR and of the Joint Secretaries are appropriate for resolving issues that might impede an assessment of the risk posed by a compound or class of compounds, particularly on general principles for risk assessments.

For compounds of mutual interest, JMPR and JECFA should have each other’s recommendations/reports available when conducting evaluations. The Joint Secretaries will have the responsibility for obtaining and distributing the documents and information, as appropriate.

The Committee agreed with this recommendation. The Joint Secretaries should ensure that for compounds already evaluated by the other group, the evaluation report is provided to the person(s) reviewing the compound, so that it is considered when the substance is evaluated.

The proposed draft definitions of commodities for Volume 3 of Residues of veterinary drugs in foods (6) are listed in Annex 3.

3. **Comments on residues of specific veterinary drugs**

The Committee considered one antimicrobial agent, four insecticides and one production aid for the first time. It reconsidered one anthelmintic agent, three antimicrobial agents and two insecticides. The recommendations made with regard to these substances and details of further studies and other information required are summarized in Annex 2.

Toxicological monographs or monograph addenda were prepared on all of the compounds on which toxicological evaluations were performed. Residue monographs were prepared on all of the substances reviewed.

3.1 **Anthelmintic agent**

3.1.1 **Ivermectin**

Ivermectin is widely used as a broad-spectrum antiparasitic drug against nematodes and arthropods in food-producing animals. In human medicine, it is used mainly for the treatment of onchocerciasis. Ivermectin is a mixture of two isomers. It contains $\geq 80\%$ of the isomer 22,23-dihydroavermectin B$_{1a}$ (ivermectin B$_{1a}$) and $\leq 20\%$ of the isomer 22,23-dihydroavermectin B$_{1b}$ (ivermectin B$_{1b}$). Ivermectin
was previously reviewed by the Committee at its thirty-sixth and fortieth meetings (Annex 1, references 97 and 104). At the latter meeting, the Committee allocated an ADI of 0–1μg/kg of body weight and recommended MRLs in cattle of 100μg/kg for liver and 40μg/kg for fat as ivermectin B₁₄. At its present meeting, the Committee reviewed a study in which the drug was applied topically to dairy cows.

Residue data
Groups of six lactating Holstein–Friesian and six lactating Jersey dairy cows were treated topically with a single dose of ivermectin of approximately 0.58mg/kg of body weight (recommended dose = 0.5mg/kg of body weight). Animals of the two breeds differed in a number of characteristics which might have influenced the depletion of residues of ivermectin in milk. For example, the Holstein–Friesian cows weighed 560–640kg and were in about the middle of the second to eighth lactation period, while the Jersey cows weighed 370–430kg and were at the beginning of the third or fourth lactation period. During the first 220h after treatment, the milk yield of the Holstein–Friesian cows was 250–310kg containing 9.9–12kg of milk fat, while that of the Jersey cows was 140–200kg containing 7.4–13kg of milk fat. During that period, the total amount of residues secreted into the milk was 0.6–1.0mg or 0.2–0.3% of the dose for the Holstein–Friesian cows and 0.7–1.4mg or 0.3–0.5% of the dose for the Jersey cows.

Milk samples from the two breeds were collected twice daily and analysed by a method with a reported limit of detection of 1μg/kg for both ivermectin B₁₄ and ivermectin B₁₅.

The concentrations of ivermectin B₁₄ in the milk of the Holstein–Friesian cows reached a maximum at the third or fourth milking after treatment; and subsequently one or more, usually broader, maxima were reached. The later maximum concentrations were typically lower than the first one, except in milk obtained from one cow in which the highest concentration was reached at the tenth milking, about 130h after treatment. In Jersey cows, the kinetics of depletion of residues of ivermectin in milk was similar, but the maximum concentrations were generally lower.

In the Holstein–Friesian cows, the highest concentrations of ivermectin B₁₄ in the milk of individual animals during the period of observation were 5–10μg/kg. In the Jersey cows, the highest concentrations of ivermectin B₁₄ in milk were 10–18μg/kg. The contribution of residues of ivermectin B₁₄ to the concentration of total residues was insignificant in both studies and typically below the reported limit of detection.
Analytical methods
A method for the identification and quantification of ivermectin B$_{1a}$ and ivermectin B$_{1b}$ residues in milk was available. It is a modification of a published method considered by the Committee at its thirty-sixth meeting (Annex 1, reference 91) and involves separation by high-performance liquid chromatography (HPLC) and detection of derivatives of the parent compounds by fluorescence. The method reviewed by the Committee at its present meeting did not conform with Good Laboratory Practice (GLP), and an incomplete set of data on its validation was made available by the sponsor. Neither the limits of detection nor the limits of quantification for the two compounds were determined. The recovery of the method was estimated in tests with milk containing ivermectin B$_{1a}$ at 5, 25 and 50μg/kg and ivermectin B$_{1b}$ at 2 and 4μg/kg; however, many of the milk samples obtained in the residue-depletion study contained lower concentrations.

Maximum Residue Limits
In reaching its decision on MRLs for ivermectin, the Committee took the following factors into account:

- The ADI of 0–1μg/kg of body weight, which is equivalent to a maximum ADI of 60μg for a 60-kg person.
- The previously recommended MRLs of 100μg/kg for liver and 40μg/kg for fat in cattle account for 39.4μg of the maximum ADI, leaving about 21μg to be ingested from milk.
- A concentration of about 10μg/kg of ivermectin B$_{1a}$ in whole milk would result in an additional 15μg of residues, based on a daily intake of 1.5 kg of milk.

The Committee recommended a temporary MRL of 10μg/kg for whole milk in cattle, expressed as ivermectin B$_{1a}$.

The Committee was aware that with the formulation of ivermectin that was reviewed, this temporary MRL would require the milk of up to 11 milkings to be discarded. The MRL could, however, serve as a basis for the development of other formulations and/or other conditions of use.

The full set of data for validation of the analytical method and information on other routes of application of ivermectin to cattle are required to evaluate the residues in milk in 2002.

3.2 Antimicrobial agents
3.2.1 Flumequine
Flumequine is a fluoroquinolone antimicrobial agent predominantly active against Gram-negative bacteria, which is used to control infections in beef cattle, sheep, pigs, chickens and farmed trout.
Flumequine was previously considered by the Committee at its forty-eighth meeting (Annex 1, reference 128), when it established an ADI of 0–30 μg/kg of body weight and recommended MRLs in cattle of 500 μg/kg for muscle, 1000 μg/kg for liver, 3000 μg/kg for kidney and 1000 μg/kg for fat, expressed as parent drug. These values would give a theoretical maximum daily intake of residues of 1100 μg, which is within the maximum ADI of 1800 μg for a 60-kg person.

In the absence of data on the contribution of parent drug to the total residues in sheep, chickens and pigs, the Committee at its forty-eighth meeting recommended temporary MRLs in these species of 500 μg/kg for muscle, 1000 μg/kg for liver, 3000 μg/kg for kidney and 1000 μg/kg for fat, expressed as parent drug. The Committee also recommended a temporary MRL of 500 μg/kg for trout muscle, including skin in normal proportions, expressed as parent drug.

At its forty-eighth meeting, the Committee requested that studies be conducted with radiolabelled flumequine in pigs, sheep, chickens and trout in order to estimate the proportion of the total residues accounted for by the parent drug. The results of these studies were required for evaluation in 2000.

Residue data

Cattle. Three male and three female beef cattle, weighing 125–135 kg, were given [14C]flumequine for 5 consecutive days at a dose of 12 mg/kg of body weight by subcutaneous injection into the neck. All of the animals were killed 18 h after the final injection. Muscle, liver, kidney, fat and tissue at the site of injection were analysed by HPLC for determination of total radiolabelled residues.

About 17% of the radiolabel in liver at the time of slaughter was associated with unmetabolized flumequine and the remainder with metabolites or bound residues. Flumequine accounted for about 80% of the total residues in muscle and kidney and for almost all of those in fat and tissue at the site of injection.

Pigs. Three male and three female pigs weighing 45–49 kg were given [14C]flumequine intramuscularly into the neck at an initial dose of 15 mg/kg of body weight, followed by nine doses of 7.5 mg/kg of body weight at 12-hourly intervals. The animals were killed 16 h after the final dose. Samples of muscle, liver, kidney, fat and tissue at the site of injection were analysed by HPLC to determine the total radiolabelled residues.

About 7% of the radiolabel in liver at the time of slaughter was associated with unmetabolized flumequine and the remainder with
metabolites or bound residues. Flumequine accounted for about 75% of the total residues in muscle and fat, and for 44% and 55% of those in kidney and skin with adhering fat, respectively.

Sheep. Three male and three female sheep weighing 38–50 kg were given [14C]flumequine intramuscularly into the neck at an initial dose of 12 mg/kg of body weight, followed by nine doses of 6 mg/kg of body weight at 12-hourly intervals. The animals were killed 16 h after the final injection. Samples of muscle, liver, kidney, fat and tissue at the site of injection were analysed by HPLC for determination of total radiolabelled residues.

About 6% of the radiolabel in liver at the time of slaughter was associated with unmetabolized flumequine and the remainder with metabolites or bound residues. Unmetabolized flumequine accounted for about 49% of the radiolabel in muscle and for 35% and 56% of that in kidney and fat, respectively.

 Chickens. Three male and three female broiler chickens weighing 2.2–2.6 kg were given [14C]flumequine by gavage into the crop at a dose of 18 mg/kg of body weight for 5 consecutive days. All of the birds were killed 12 h after the final dose. Samples of muscle, liver, kidney, fat and skin with adhering fat were analysed by HPLC for determination of total radiolabelled residues.

Unmetabolized flumequine accounted for about 94% of the radiolabel in muscle and for about 70%, 76%, 100% and 77% of that in liver, kidney, fat and skin with adhering fat, respectively.

Trout. Two groups of 20 rainbow trout with average weights of 90 g and 100 g were maintained in separate tanks with water temperatures of 7 °C and 16 °C, respectively. A single dose of [13C]flumequine was administered by gavage as a 2% formulation in lactose enclosed in a gelatine capsule at a dose of about 12 mg/kg of body weight. The doses received by individual fish were calculated from their weight at slaughter. Five trout from the group maintained at 16 °C were slaughtered 18 h after treatment and five 36 h after treatment, and five trout from the group maintained at 7 °C were slaughtered at 36 h and five at 96 h.

The results of HPLC and radiochemical analyses at the time of slaughter showed no detectable metabolism of flumequine at any time at either temperature.

Analytical methods

An HPLC method with fluorescence detection for determining flumequine and its metabolite, 7-hydroxyflumequine, was used in
the residue-depletion studies evaluated by the Committee at its forty-eighth meeting. The method evaluated by the Committee at its present meeting did not include an enzymatic hydrolysis step and had only been validated for flumequine.

The flumequine residues were extracted with ethyl acetate, and iba-floxacin was added to the sample extract after purification by liquid–liquid extraction to check the retention time. An extra step was added for all samples with a high fat content, in which the extract was partitioned between acetonitrile and hexane. Separation and quantification of flumequine were achieved by HPLC on a C18 column with gradient elution involving various mixtures of acetonitrile and aqueous oxalic acid at a concentration of $2.7 \times 10^{-3}$ mol/l. Quantification was achieved using a fluorescence detector and by comparison of the results with a calibration curve constructed from data obtained by analysis of tissue samples fortified with flumequine. The linearity, accuracy, repeatability, and limits of detection and quantification of the method were tested in a single laboratory and found to be acceptable. The limit of quantification of the method was 50 μg/kg for all tissues, and the limit of detection was 10–25 μg/kg. The recovery was greater than 75% for all tissues.

The Committee was aware that a number of suitable analytical methods are available for measuring flumequine residues in the edible tissues of food-producing animals.

Maximum Residue Limits

In reaching its decision on MRLs for flumequine, the Committee took the following factors into account:

- The ADI of 0–30 μg/kg, which corresponds to a maximum ADI of 1800 μg for a 60-kg person.
- On the basis of the residue-depletion study in cattle treated with radiolabelled flumequine, the parent drug accounted for 79–100% of the total residues in muscle, kidney and fat. These values are somewhat greater than the value of 50% estimated from the studies reviewed by the Committee at its forty-eighth meeting. The parent drug also accounted for 17% of the total residues in liver, which is lower than the value of 25% estimated from the studies reviewed at the forty-eighth meeting.
- On the basis of the residue-depletion studies in sheep treated with radiolabelled flumequine, the parent drug accounted for 40% (range, 35–56%) of the total residues in muscle, kidney and fat, and for 6% of those in liver.
- On the basis of the residue-depletion studies in pigs treated with radiolabelled flumequine, the parent drug accounted for 59%
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (μg/kg)</th>
<th>Estimate of total residues (μg/kg)</th>
<th>Theoretical maximum daily intake (μg flumequine equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>500</td>
<td>1250*</td>
<td>375</td>
</tr>
<tr>
<td>Liver</td>
<td>500</td>
<td>8300*</td>
<td>830</td>
</tr>
<tr>
<td>Kidney</td>
<td>3000</td>
<td>7500*</td>
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<tr>
<td>Fat</td>
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<td>2500*</td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1705</td>
</tr>
</tbody>
</table>

* Expressed as parent drug.

Flumequine undergoes less metabolism in chickens than in mammalian species. On the basis of the residue-depletion studies in chickens treated with radiolabelled flumequine, the parent drug accounted for 82% (range, 70–94%) of the total residues.

- There were no measurable residues of flumequine metabolites in trout.
- Suitable analytical methods are available for measuring flumequine residues.

The Committee recommended MRLs for flumequine of 500μg/kg for muscle and liver, 3000μg/kg for kidney and 1000μg/kg for fat in cattle, pigs, sheep and chickens, expressed as parent drug. The Committee also recommended an MRL of 500μg/kg for trout muscle with skin in normal proportions.

From the values for the MRLs for sheep, the theoretical maximum daily intake of flumequine residues would be approximately 1700μg (Table 1). This would be within the maximum ADI of 1800μg for a 60-kg person.

### 3.2.2 Lincomycin

Lincomycin belongs to the galacto-octopyranoside class of antibiotics known as lincomamides, which also includes pirlimycin and clindamycin. It is mainly active against Gram-positive bacteria and acts on the 50S subunit of the ribosome to inhibit RNA-dependent protein synthesis. Lincomycin is used alone or in combination with other antimicrobials such as spectinomycin, neomycin, sulfadiazine and sulfadomidine. It can be given orally in feed or drinking-water, by intramuscular injection, or as an intramammary infusion.
The recommended doses are: 0.5 mg/kg of body weight in feed and 3–50 mg/kg of body weight in drinking-water for poultry; 0.2–13 mg/kg of body weight in feed, 5–10 mg/kg of body weight in drinking-water, and 5–10 mg/kg of body weight intramuscularly in pigs; 5 mg/kg of body weight intramuscularly in calves and sheep; and 200–330 mg/quarter of the udder as an intramammary infusion, three times after each milking, in dairy cows.

The Committee has not previously evaluated lincomycin.

Toxicological data
The Committee considered data from a range of toxicological studies on lincomycin, including the results of studies on its pharmacokinetics, metabolism, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity, ototoxicity, immunotoxicity, reproductive toxicity, developmental toxicity and microbiological safety. The results of studies on the functionally and structurally related drug clindamycin were considered in the assessment of the microbiological safety of lincomycin. In all of the studies considered, the concentrations of the compound were reported in terms of the activity of lincomycin base. While many of the studies were conducted prior to the development of GLP, all of the pivotal studies were carried out according to appropriate standards for study protocol and conduct.

Dogs given lincomycin intramuscularly or orally showed rapid absorption, peak serum concentrations being achieved within 0.5 and 1.5 h, respectively. In pigs given an oral dose, 53% (with a standard deviation of 19%) was bioavailable, and 5–15% was bound to plasma proteins. The peak concentrations in serum were reached within 3.6 h (standard deviation, 1.2 h), with a half-life of 3.4 h (standard deviation, 1.3 h). Pig excreta contained little unchanged lincomycin: urine contained 11–21% of the oral dose, half of which was unchanged parent compound. Only trace amounts of N-desmethylincomycin were identified. The contents of the gastrointestinal tract accounted for 79–86% of the excreted drug. In faecal samples, only 17% of the excreted dose was unchanged parent compound, and the remainder consisted of uncharacterized metabolites.

Lincomycin is well distributed in the human body. Significant concentrations were found in the bile, pleural fluid, brain, bone marrow, synovial fluid, bone, joint capsules, eyes and peritoneal fluid following oral administration. The distribution of the compound in cerebrospinal fluid is generally poor, except in the presence of inflammation. Lincomycin has been shown to cross the placenta, and peak concentrations of 0.2–3.8 μg/ml were found in amniotic fluid, which were
sustained for 52 h after a single intramuscular injection of 600 mg
to pregnant women. Lincomycin was present in the milk of these
women. The systemic oral bioavailability of lincomycin in persons
who had fasted was 25–50%, but this value can be as low as 5% in
the presence of food; 72% of the amount found in serum was bound
to serum proteins. Peak serum concentrations are usually reached
within 4 h, with a half-life of 4.2–5.3 h.

Lincomycin is extensively metabolized, as less than 10% of the dose
was present as unchanged parent drug in animal tissues. The numer-
ous metabolites include N-desmethyllincomycin and lincomycin sul-
foxide, which are reported to have 1–7% of the microbiological activity
of the parent compound.

Lincomycin has high acute toxicity only in rabbits. The LD₅₀ in mice
and rats treated orally was 17–19 and 11–16 g/kg of body weight
respectively, while the lethal dose in 9 of 15 rabbits was reported to be
50 mg/kg of body weight.

Lincomycin was administered to mice for 90 days in the feed to
provide a dose of 0, 10, 30, 100, 300 or 3000 mg/kg of body weight per
day. Animals given the two highest doses showed increased weight of
the intestine (and pancreas) and an increased incidence of luminal
distension and dilatation of the small and large intestine. The NOEL
was 100 mg/kg of body weight per day.

In a 90-day toxicity study in dogs, groups of four males and four
females were given lincomycin orally at 0, 400 or 800 mg/kg of body
weight per day. Transient increases in serum alanine aminotrans-
ferase activity were observed during the first month of treatment in all
dogs in the highest-dose group and in one dog in the group treated at
400 mg/kg of body weight per day, but the activity had returned to
normal by the end of the study. No other treatment-related effects
were reported. The NOEL was 800 mg/kg of body weight per day, the
highest dose tested.

Groups of 10 rats of each sex were treated for 1 year with lincomycin
at a dose of 0, 30, 100 or 300 mg/kg of body weight per day by oral
gavage. No treatment-related effects were reported at any dose.
The NOEL was 300 mg/kg of body weight per day, the highest dose
tested.

In a study that did not conform to GLP, lincomycin was administered
to groups of two male and two female dogs in capsules for 6 months
at a dose of 0, 30, 100 or 300 mg/kg of body weight per day. The NOEL
was 300 mg/kg of body weight per day, the highest dose tested. In a 1-
year study, groups of five male and five female dogs were given gelatin
capsules containing premix-grade lincomycin at a dose of 0, 0.38, 0.75 or 1.5 mg/kg of body weight per day or United States pharmacopoeia (USP)-grade lincomycin at a dose of 1.5 mg/kg of body weight per day. The NOEL was 1.5 mg/kg of body weight per day, the highest dose tested.

In a long-term study of toxicity and carcinogenicity conducted according to GLP, pregnant female rats and groups of 60 offspring of each sex were given feed containing premix-grade lincomycin to provide a dose of 0, 0.38, 0.75 or 1.5 mg/kg of body weight per day or USP-grade lincomycin to provide a dose of 1.5 or 100 mg/kg of body weight per day. Treatment of the offspring was continued for 26 months. No treatment-related effects were reported. While lincomycin was not carcinogenic under the conditions of the assay, the Committee considered the administered dose to be insufficient for assessing the carcinogenicity of lincomycin. The NOEL for non-neoplastic lesions was 100 mg/kg of body weight per day, the highest dose tested.

Lincomycin was tested in vitro for its capacity to induce reverse mutations in bacteria, gene mutations in Chinese hamster lung fibroblasts, unscheduled DNA synthesis in primary rat hepatocytes, chromosomal aberrations in peripheral human lymphocytes, DNA damage in Chinese hamster V79 cells and micronuclei in rat and mouse bone marrow, and in vivo for its ability to induce sex-linked recessive lethal mutations in Drosophila melanogaster. The only positive finding was the induction of unscheduled DNA synthesis in primary rat hepatocytes, but this result could not be replicated.

Adequate studies of carcinogenicity were not available. However, the weight of the evidence indicates that lincomycin is not genotoxic, and lincomycin is not structurally similar to known carcinogens. The Committee therefore concluded that the drug does not present a carcinogenic risk, and further carcinogenic studies were deemed unnecessary.

The reproductive and developmental toxicity of lincomycin was evaluated in a three-generation study conducted prior to the formulation of GLP. Groups of 30 male and 60 female F₀ rats and 10 male and 20 female F₁, F₂ and F₃ animals were given diets containing premix-grade lincomycin to provide a dose of 0, 0.38, 0.75 or 1.5 mg/kg of body weight per day or USP-grade lincomycin to provide 1.5 or 100 mg/kg of body weight per day, beginning with F₀ weanling rats and continuing through successive breeding of the F₀, F₁ and F₂ progeny to weaning of the F₃ litters. No treatment-related effects were seen at any dose. The NOEL was 100 mg/kg of body weight per day for the USP-grade lincomycin, the highest dose tested.
In a two-generation study that was conducted according to GLP, lincomycin was administered to groups of 30 male and 30 female rats by gavage at a dose of 0, 100, 300 or 1000 mg/kg of body weight per day. No treatment-related effects were observed at any dose. The NOEL was 1000 mg/kg of body weight per day, the highest dose tested.

In a study of developmental toxicity which did not conform to GLP, pregnant rats were given lincomycin by gastric gavage at a dose of 0, 10, 30 or 100 mg/kg of body weight per day on days 6–15 of gestation. An increased incidence of fetal resorptions was observed at the highest dose. The NOEL for embryotoxicity was 30 mg/kg of body weight per day.

The potential ototoxicity of lincomycin was tested in groups of three cats that received intramuscular injections of 30 or 60 mg/kg of body weight per day for 2.5 months. Hearing and vestibular function were evaluated. No treatment-related effects were reported.

*Microbiological data*

The Syrian hamster is used as an experimental model to evaluate antibiotic-associated colitis. In studies in which lincomycin was administered by various parenteral routes to hamsters, the NOEL for antibiotic-associated colitis was 0.1 mg/kg of body weight per day.

Administration of lincomycin at an oral therapeutic dose of 25–66 mg/kg of body weight daily to 12 patients for periods varying from 6 to 150 days caused antibiotic-associated colitis. Administration of the structurally and functionally similar compound clindamycin to 10 patients for 7 days at a dose of 10 mg/kg of body weight per day also resulted in antibiotic-associated colitis. When clindamycin was administered to 99 patients at doses of up to 2.5 mg/kg of body weight per day for up to 12 months, the NOEL for adverse effects on the gastrointestinal microflora was 2.5 mg/kg of body weight per day.

The ability of lincomycin to affect faecal excretion of pathogens was investigated in a study of 32 pigs aged 4–5 weeks that were given the drug for periods of up to 45 days at a concentration of 100 g/t of feed, equal to a dose of 5.6 mg/kg of body weight per day. Comparison of these animals with pigs that received the vehicle alone showed that treatment had no effect on the faecal excretion of *Salmonella typhimurium*, or on its susceptibility to lincomycin.

The sensitivity of staphylococci isolated from pigs and poultry to lincomycin was investigated in a 10-year study that ended in 1980. No change in the susceptibility of *Staphylococcus aureus* strains isolated was observed.
A study was conducted between 1971 and 1982 to examine the patterns of susceptibility to antibiotics of more than 5 million bacterial strains from hospitals across the USA. The susceptibility of Gram-positive aerobic and anaerobic bacteria to lincomycin changed little during the survey period. In a separate study, the median microbiological inhibitory concentration (MIC$_{50}$) for representative bacteria from the human gut was reported for lincomycin. The no-observed-effect concentration (NOEC) for the effect of lincomycin on *Fusobacterium*, the most sensitive representative species, was 0.2 µg/ml.

Clindamycin was tested at a concentration of 0, 0.26, 2.6, 25 or 260 µg/ml of culture for 7 days in semi-continuous cultures of composite faecal samples from humans. During these treatments and for 7–8 days thereafter, *Clostridium difficile* was added daily at a concentration of 10$^5$ cells/ml. The NOEL for clindamycin was 2.6 µg/ml on the basis of overgrowth of *C. difficile*, changes in pH, and changes in the concentration of volatile fatty acids in the culture medium.

A decision-tree for evaluating the potential of veterinary drug residues to affect the human intestinal microflora was developed by the Committee at its fifty-second meeting (Annex 1, reference 140; Fig. 1). At its present meeting, the Committee used the decision-tree to evaluate the potential of lincomycin residues to affect the human intestinal flora:

- **Does the ingested residue have antimicrobial properties?**
  
  Yes.

- **Does the drug residue enter the lower bowel?**
  
  Yes.

- **Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, metabolism mediated by the host, or by the intestinal microflora in the bowel and/or by binding to intestinal contents?**
  
  Yes, but microbiologically active residues remain.

- **Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal microflora?**
  
  No.

- **Do clinical data from therapeutic use of the class of drugs in humans or from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal microflora?**
  
  Yes.
• **Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora.**

The available data indicate that disruption of the barrier to colonization of the human gastrointestinal microflora is the major concern, rather than the emergence of drug-resistant populations. Lincosamides are used widely in human medicine and have been shown to disrupt the intestinal microflora. In a study of the magnitude of and trends in the development of bacterial resistance to lincosamides, the pattern of susceptibility of human isolates of Gram-positive aerobic and anaerobic bacteria changed little over a 12-year period (1971–83). Resistance has been shown to develop in staphylococci isolated from humans or animals, but most isolates remain susceptible to lincomycin.

While no data were available on the effects of lincomycin on the metabolic activity of the intestinal microflora, the Committee concluded that disruption of the barrier to colonization of the gastrointestinal microflora is the most appropriate microbiological end-point for determining an ADI for lincomycin.

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

Disruption of the barrier to colonization of the human gastrointestinal microflora is the microbiological end-point of concern with lincomycin. No studies suitable for deriving a NOEL for the effects of lincomycin on the gastrointestinal microflora in humans were available. However, clindamycin has the same spectrum of activity and the same reported spectrum of adverse effects in humans as lincomycin, and is generally considered to be a more potent antibacterial agent than lincomycin. Data were available to show that the availability to the colon of orally administered clindamycin is one-tenth of that of lincomycin. The Committee concluded that the study of the clinical effects of orally administered clindamycin in humans is the most appropriate one for determining the microbiological safety of lincomycin.

The Committee could have established an ADI of 0–300μg/kg of body weight for lincomycin, based on the NOEL of 30mg/kg of body weight per day for embryotoxicity in rats and a safety factor of 100. The Committee noted, however, that lincomycin belongs to a group
Figure 1
Decision-tree for determining the potential adverse effects of residues of veterinary antimicrobial drugs on the human intestinal microflora

1. Assess the effects of veterinary drug residues, including metabolites, on the human intestinal microflora.

   Yes: Does the ingested residue have antimicrobial properties (recommendation 1(a))?

   No

   2. Does the drug residue enter the lower bowel (e.g. with the food bolus, by biliary circulation and/or by mucosal secretion) (recommendation 1(b))?

   Yes: Conclude that the drug residue will not affect the intestinal microflora and use toxicological data to derive the ADI (recommendation 1(a)).

   No

   3. Is the ingested residue transformed irreversibly to inactive metabolites by chemical transformation, metabolism mediated by the host or by the intestinal microflora in the bowel and/or by binding to intestinal contents (recommendations 1(b)–1(d))?

   Yes

   4. Conclude that the drug residue will not affect the intestinal microflora and use toxicological data to derive the ADI (recommendations 1(c) and 1(d)).

   No

   5. Do data on the effects of the drug on the colonic microflora provide a basis to conclude that the ADI derived from toxicological data is sufficiently low to protect the intestinal microflora (recommendation 1(e))?

   Yes: Conclude that the drug residue will not affect the gastrointestinal microflora and use toxicological data to derive the ADI (recommendation 1(e)).

   No

   6. Do clinical data from therapeutic use of the class of drugs in humans or data from in vitro or in vivo model systems indicate that effects could occur in the gastrointestinal microflora (recommendation 1(f))?

   Yes

   7. Determine the most sensitive adverse effect(s) of the drug on the human intestinal microflora, including selection of drug-resistant populations, disruption of the barrier to colonization or changes in the metabolic activity of the microflora in the gastrointestinal tract that have been specifically linked to adverse effects on human health.

   No: Conclude that the drug residue will not affect the gastrointestinal microflora and use toxicological data to derive the ADI.
If emergence of antimicrobial resistance is the concern, conduct tests in vitro (continuous culture of faecal inocula) or in vivo (rodents inoculated with human flora); challenge the model system with an antibiotic-resistant species and determine the concentration of the drug that does not select for resistance or the antibiotic-resistant strain when compared with absence of the drug. Use the dose of the drug that has no effect to derive an ADI (recommendation 2(d)).

If disruption of the colonization barrier is the concern, determine the MIC of the drug against 100 strains of the predominant intestinal flora and take the geometric mean MIC of the most sensitive genus or genera to derive an ADI using the equation\textsuperscript{*} discussed at the forty-seventh meeting of the Committee (Annex 1, reference 125). Other model systems may be used to establish a no-observed-effect concentration (NOEC) to derive an ADI (recommendation 2(b)). A more realistic ADI can be derived by conducting tests in vitro (continuous culture of faecal inocula) or in vivo (rodents inoculated with human flora). Challenge the model systems with appropriate species (e.g., *Clostridium difficile*, *Salmonella* spp., *Enterococcus* spp., *Escherichia coli*) and determine the concentration of the drug that does not alter the shedding characteristics of the organisms when compared with absence of the drug. Use the dose of the drug that has no effect to derive an ADI (recommendation 2(c)).

If the concern is change in a specific enzymatic activity that is directly linked to adverse effects on human health, conduct tests in vitro (continuous culture of faecal inocula) or in vivo (rodents inoculated with human flora) to determine the concentration of the drug that does not alter that specific enzymatic activity when compared with absence of the drug. Use the dose of the drug that has no effect to derive an ADI (recommendation 2(e)).

\textsuperscript{*} The equation is as follows:

\[
\text{Upper limit of ADI (\mu g/kg of body weight)} = \frac{\text{MIC(\mu g/g)} \times \text{Mass of colonic contents (g)}}{\text{Fraction of oral dose bioavailable} \times \text{Safety factor} \times \text{Weight of human (kg)}}
\]

where:

MIC\textsubscript{\text{90}} = Minimum concentration of an antimicrobial drug completely inhibiting the growth of 50\% of the cultures of a particular microorganism, as judged by the naked eye, after a given period of incubation. For the purpose of the evaluation, the MIC\textsubscript{\text{90}} value is the mean MIC\textsubscript{\text{90}} for the strain(s) of the relevant species tested. Alternatively, the lowest MIC\textsubscript{\text{90}} value for the most sensitive species can be used.

Although MIC\textsubscript{\text{90}} values are usually expressed in \mu g/ml, they are expressed as \mu g/g in this equation so that the ADI will be in \mu g/kg. When the MIC\textsubscript{\text{90}} value is converted to these units, it is assumed that the density of the experimental medium is 1 g/ml.

A value of 220 g is used for the mass of the colonic contents and a value of 60 kg is used for the weight of an adult. The safety factor used to take account of uncertainty about the amount and relevance of data available for review may range from 1 to 10. A value of 1 is used when extensive relevant microbiological data are provided.
of lincosaminides that is active against Gram-positive bacteria and that the human gastrointestinal flora are sensitive to therapeutic doses of this group of compounds. Because this is the most sensitive end-point, the Committee established an ADI of 0–30μg/kg of body weight for lincomycin, based on the NOEL of 2.5mg/kg of body weight per day for the effects of clindamycin on the gastrointestinal microflora¹ and a safety factor of 100.² The Committee rounded the value of the ADI to one significant figure, in accordance with usual practice.

**Pharmacokinetic and metabolism data**

The Committee considered a number of studies on the metabolism and pharmacokinetics of lincomycin, including several studies in pigs in which the drug was administered by intravenous, oral and intra-muscular routes.

Lincomycin is rapidly absorbed after parenteral or oral administration in all food animal species and is widely distributed to tissues. It is extensively metabolized to compounds that are reported to have no or minimal antimicrobial activity.

In a study in pigs, seven animals were treated intravenously with lincomycin hydrochloride at 10mg/kg of body weight, followed 7 days later by the same dose given orally. After the intravenous treatment, elimination of the drug followed a biphasic, two-compartment model with a mean half-life of 2h. After the oral treatment, 53% ± 19% of the dose was absorbed and 5–15% was estimated to be bound to the plasma proteins at blood concentrations of 0.5–20mg/kg. The absorption and bioavailability of the drug after oral administration followed first-order kinetics, and the distribution and excretion followed a one-compartment model and first-order elimination with a mean half-life of 3.4h. The concentration of lincomycin in the blood reached a maximum of 1.45mg/kg at 3.6h after oral treatment. The wide variation found in the maximum concentration was attributed to the effect of different food intakes.

¹ The observed NOEL was 2.5mg/kg of body weight per day. While this was the highest dose administered, a separate trial showed an effect on the gastrointestinal microflora of clindamycin at 10mg/kg of body weight per day and of lincomycin at 25mg/kg of body weight per day.

² The overall safety factor was 100, based on a safety factor of 10 to address variation among individuals and a correction factor of 10 to address the higher oral availability to the colon of lincomycin compared to clindamycin.
In a study in which $^{[14]}C$lincomycin hydrochloride was administered orally to pigs, the highest concentrations of radiolabel were found in liver and kidney, with much lower concentrations in muscle and fat. In two groups of pigs which received unlabelled lincomycin hydrochloride intramuscularly at 1 mg/kg of body weight per day for either 3 or 7 days, the highest concentrations were found in urine, consistent with very rapid excretion. The tissue concentrations were highest at the injection sites, followed by kidney, liver, muscle and fat.

In dairy cattle given lincomycin intravenously at 5.5 or 11 mg/kg of body weight, analysis of blood, milk and urine samples showed that the elimination of the drug followed first-order kinetics, with about 32% of the dose excreted in the urine. However, whereas only about 1.5% of an intravenous dose was excreted into the milk, analysis of blood samples from one cow given an intramammary infusion at 11 mg/kg of body weight showed that 85% of the dose was absorbed into the blood. Approximately 65% of the dose was metabolized to inactive metabolites, regardless of the route of administration.

In a pharmacokinetic study in chickens, eight birds received unlabelled lincomycin hydrochloride in the diet at 10 mg/kg of feed for 36 days, followed by $^{[14]}C$lincomycin hydrochloride at 0.47–0.76 mg/kg of body weight orally twice daily for 12 days. During treatment, 90% of the radiolabel was found in excreta. The half-life in bile and offal was 8.3 and 11.3 h, respectively. Only liver samples collected at 1 h after treatment contained detectable residues (limit of detection, 0.1 mg/kg) and these were biologically inactive.

In pigs, lincomycin was metabolized rapidly and extensively; 26 metabolites were found in liver. Except for the parent compound, none of the metabolites was characterized, and none accounted for more than 10% of the total radiolabelled residues. In a comparative study of a microbiological method and a gas chromatography–mass spectrometry (GC–MS) method, lincomycin appeared to account for all of the microbiologically active residues in pig liver and kidney.

In comparative studies in pigs and rats and in chickens and rats, the metabolism of lincomycin was found to be qualitatively similar, although the metabolites were not identified.

In chickens given a dose of about 7 mg/kg of body weight per day in drinking-water for 7 days, the liver and kidney contained the highest concentrations of total residues. In liver immediately after withdrawal of the medicated drinking-water, lincomycin accounted for 20% of the total residues and lincomycin sulfoxide, $N$-desmethylincomycin and $N$-desmethylincomycin sulfoxide accounted for 40%, 5% and
10%, respectively. The remaining residues were not identified. In muscle, lincomycin accounted for 16% of the total residues and an unidentified metabolite for 37%. In skin with adhering fat immediately after withdrawal of the drug, lincomycin accounted for 18% of the total residues and the same unidentified metabolite for 11%. In excreta, lincomycin accounted for 60–85% of the total residues during treatment and 50–55% of the total residues 4 days after treatment. Of the remaining residues found in excreta during treatment, 6–10% were identified as lincomycin sulfoxide, 3–6% as N-methyl lincomycin, and 10% as an unknown metabolite.

Residue data

Cattle. Three studies were conducted according to GLP in which lincomycin was administered by intramammary infusion. One study in which the highest recommended dose was used involved 24 cows that received three consecutive doses of 330 mg of lincomycin into each of the four quarters of the udder at 12-h intervals. Pooled milk samples were taken at 12-h intervals for eight milkings after the last treatment and analysed by GC–MS. The mean concentrations of lincomycin in milk were 53 mg/kg at 12h, 7.0 mg/kg at 24h, 0.7 mg/kg at 36h, 0.2 mg/kg at 48h, 0.04 mg/kg at 60h, and below the limit of quantification (0.015 mg/kg) at all other times.

In another study that complied with GLP, 16 cows were given three consecutive intramammary infusions of 330 mg of lincomycin into each of the four quarters of the udder at 12-h intervals. Tissue samples from groups of four cows killed 1, 7, 14 and 21 days after treatment were analysed by GC–MS. The mean concentrations of lincomycin residues in liver were 0.23 mg/kg on day 1 and 0.06 mg/kg on day 7, and ranged from 0.02 to 0.04 mg/kg on day 14 and from below the limit of quantification (0.02 mg/kg) to 0.05 mg/kg on day 21. Residues were found in muscle and kidney only on day 1, and no residues were found in fat.

In a study conducted prior to the development of GLP, five lactating cows received three consecutive infusions of 200 mg of lincomycin into one quarter of the udder at 12-h intervals. Milk samples taken during treatment and at 12-h intervals for 10 milkings after treatment were analysed by a microbiological assay. The mean concentrations of residues in milk decreased from 115 mg/kg at 12h to 18 mg/kg at 24h, 1.4 mg/kg at 36h, and below the limit of quantification (0.2 mg/kg) at 48h.

Veal calves. In a study conducted according to GLP, four groups of five veal calves weighing 60–80 kg received daily intramuscular injections of
5 mg of lincomycin for 5 days, two doses being given on day 1 on both sides of the neck. Animals were killed after 8 h and on days 7, 14 and 21 after the last treatment, and tissue samples were taken and analysed by GC–MS. At 8 h, the highest mean concentrations of residues were found in kidney (3.3 mg/kg) and at the site of the last injection (2.4 mg/kg). The mean concentration of residues in muscle was 0.72 mg/kg. The concentrations ranged from below the limit of quantification (0.02 mg/kg) to 0.14 mg/kg in liver and from below the limit of quantification to 0.26 mg/kg in fat at 8 h. The only other sample in which residues were detected was a liver sample taken on day 14; a concentration of 0.072 mg/kg was found.

*Pigs.* Several residue-depletion studies in pigs treated orally or intramuscularly with lincomycin were evaluated. In a study conducted prior to the development of GLP, four groups of six pigs were fed diets containing [14C]lincomycin at concentrations equivalent to 1.2, 2.0, 6.0–7.0 or 10–12 mg/kg of body weight per day for 3 days and were killed 12 h after the last treatment. An additional group of six pigs received 10–12 mg/kg of body weight per day for 3 days and was killed 48 h after treatment. The mean concentrations of total residues in tissue samples from the four treated groups at 12 h were 0.01, 0.02, 0.05 and 0.15 mg/kg in muscle; 0.40, 0.64, 1.6 and 3.4 mg/kg in liver; 0.22, 0.41, 1.2 and 3.1 mg/kg in kidney; and 0.02, 0.02, 0.13 and 0.35 mg/kg in fat, respectively. At 12 h, the mean concentrations of microbiologically active residues in liver and kidney samples from these animals were 0.01 mg/kg and 0.42 mg/kg, respectively.

For the pigs dosed at 10–12 mg/kg of body weight and killed at 48 h after withdrawal, the mean concentrations of total residues were 0.09 mg/kg in muscle, 0.82 mg/kg in liver, 0.64 mg/kg in kidney and 0.10 mg/kg in fat. When the liver samples from these animals were reanalysed using an improved microbiological assay and by GC–MS, lincomycin accounted for 6% of the total residues at 12 h and 25% at 48 h after treatment.

In a study conducted according to GLP, 12 pigs received an intramuscular dose of 11 mg/kg of body weight of [14C]lincomycin daily for 3 days. Two groups of three pigs were killed at 12 and 24 h and six pigs were killed at 48 h after treatment. A total of 78–85% of the administered radiolabel was recovered. The mean concentrations of total residues were highest in liver at 12 h (17 mg/kg), followed by kidney (12 mg/kg), the injection site (1.0 mg/kg), fat (0.59 mg/kg) and muscle (0.39 mg/kg). By 48 h, the mean concentrations of total residues had declined to 3.8 mg/kg in liver, 3.1 mg/kg in kidney, 0.58 mg/kg at the injection site, 0.20 mg/kg in fat and 0.14 mg/kg in muscle. When the liver and kidney
samples were analysed using an improved microbiological assay and by GC-MS, the parent drug accounted for 14% of the total residues at 12 h, 3% at 24 h and 1.6% at 48 h in liver, and 55% at 12 h, 20% at 24 h and 7% at 48 h in kidney.

The most relevant residue-depletion study, which was conducted according to GLP, involved two groups of 24 pigs given the maximum recommended intramuscular dose (11 mg/kg of body weight) of one of two formulations of lincomycin for 3 days. The animals were killed 3, 6, 12, 24, 48 and 144 h after treatment. Samples of muscle, liver, kidney, fat and tissue from the site of injection were taken from the pigs in each group and analysed by GC-MS. The mean concentrations of residues decreased rapidly between 3 and 48 h after administration of the two formulations, from 6.4 and 4.7 mg/kg to 0.06 and 0.07 mg/kg in liver and from 29 and 21 mg/kg to 0.17 and 0.24 mg/kg in kidney, respectively. In both tissues, the concentrations were below the limit of quantification (0.02 mg/kg) at 144 h. In muscle, the mean concentrations were 3.6 and 2.6 mg/kg at 3 h, 0.06 and 0.09 mg/kg at 24 h, and below the limit of quantification at all other times. In fat, the mean concentrations were 0.47 and 0.47 mg/kg at 3 h, 0.02 and 0.03 mg/kg at 24 h, and below the limit of quantification at all other times. In tissue from the injection site, the mean concentrations were 115 and 250 mg/kg at 3 h, 0.02 and 0.03 mg/kg at 48 h, and below the limit of quantification at 144 h.

Sheep. In a study conducted according to GLP, four groups of five sheep received daily intramuscular doses of lincomycin at 5 mg/kg of body weight for 3 days. Animals were killed at 8 h and at days 7, 14 and 21 after treatment, and samples of muscle, liver, kidney and tissue at the site of injection were analysed by GC-MS. At 8 h after the last treatment, the mean concentrations of lincomycin were highest at the site of injection (14 mg/kg), followed by kidney (9.0 mg/kg) and liver (4.3 mg/kg); the lowest concentration was found in muscle (0.95 mg/kg). By day 7, only two of five samples of liver contained concentrations of residues above the limit of quantification of the method.

Chickens. In a study conducted according to GLP, groups of 21 female and 21 male 35-day-old broiler chickens were given [14C]lincomycin at doses of 5.1–6.6 mg/kg of body weight per day in their drinking-water for 7 days. Total residues were measured in muscle, liver, kidney and skin with adhering fat immediately and 0.5, 1, 2, 4 and 7 days after withdrawal of the medicated water. Liver and kidney contained the highest concentrations, but these decreased between day 0 and day 7 from 1.6 mg/kg to the limit of quantification of the analytical method (0.02 mg/kg) in liver and from 1.3 to
0.01 mg/kg in kidney. Lincomycin accounted for 20% of the total radiolabel in liver at 0h, 12% at 12h, 8% at 24h, 2% at 48h, and 5% at 96h. The mean concentrations in muscle decreased from 0.05 mg/kg at 0h to below 0.005 mg/kg at day 2, and those in skin with adhering fat from 0.13 mg/kg at 0h to below 0.005 mg/kg at day 7. Immediately after the last treatment, the radiolabel in muscle accounted for 16% of the administered dose and that in skin with adhering fat for 18%.

In a study conducted according to GLP, 18 laying hens were given gelatin capsules containing [14C]lincomycin at a dose equivalent to 0.5 mg/kg of body weight per day twice daily for 12 days. Eggs were collected on days 1–3 of treatment, and tissues were collected from six birds 4, 28 and 76h after treatment. The mean concentration of total residues in eggs rose from 0.002 mg/kg at day 1 to 0.008 mg/kg at day 10 during treatment and decreased to 0.005 mg/kg by day 2 after treatment. The mean concentrations of total residues were highest in kidney (0.15 mg/kg) and liver (0.14 mg/kg) and at the limit of quantification (0.02 mg/kg) in muscle and skin with adhering fat 4h after treatment. The concentrations of residues in all tissues were below 0.01 mg/kg 76h after treatment.

*Effect of lincomycin on starter cultures in milk processing*

The effect of lincomycin on the performance of bacterial starter cultures used for the production of Italian cheese, yoghurt, buttermilk and sour cream was investigated. The four-parameter Weibull growth curve was used to model the change in pH as a function of time. No significant effects were observed with concentrations of lincomycin of up to 0.16 mg/kg.

*Analytical methods*

Several methods have been used at different times to determine the concentrations of lincomycin residues in animal tissues and food products. The methods include a microbiological assay, thin-layer chromatography–bioautography, gas chromatography with an alkaline flame detector and GC–MS.

A microbiological method with *Micrococcus luteus* has been described for monitoring the presence of lincomycin in animal tissues and milk. The limit of quantification was reported to be 0.02–0.2 mg/kg.

GC–MS methods have been used to study the depletion of lincomycin residues from the tissues of cattle, pigs and sheep, and data on validation of these methods were provided for these applications and for monitoring lincomycin residues in chicken tissues. These methods
involves extensive sample purification, followed by derivatization and GC–MS analysis in the electron impact mode, with detection of the ion fragment at m/z 126 atomic mass units. The recovery of lincomycin by these methods was 84–102%, depending on the tissue, with a limit of quantification of 0.02–0.06 mg/kg of tissue from cattle, pigs, sheep and chickens. The coefficient of variation of the method was consistently less than 10%.

For confirmation of lincomycin residues in pig liver, GC–MS is used in the chemical ionization mode with methane as the reactant gas. The ions analysed are the derivatized molecular ion fragment at m/z 575 atomic mass units and three fragments at m/z 126, 515 and 527 atomic mass units.

Maximum Residue Limits
In recommending MRLs for lincomycin, the Committee took the following factors into account:

- An ADI of 0–30 μg/kg of body weight was established by the Committee on the basis of a microbiological end-point. This would result in a maximum ADI of 1800 μg of the parent drug and/or its equivalents for a 60-kg person.
- In pig tissues, lincomycin is the major microbiologically active residue.
- In milk, lincomycin accounts for 90% of the total residues.
- There was insufficient evidence that lincomycin is the major microbiologically active residue in cattle and sheep tissues and in hens’ eggs.
- The parent drug is the marker residue.
- Kidney and liver contain the highest concentrations of residues.
- A validated GC–MS analytical method is available which could be used routinely in many laboratories. It has a limit of quantification of 0.02–0.06 mg/kg in tissues of cattle, calves, pigs, sheep and chickens.
- Lincomycin has no effect on bacterial starter cultures used in the production of milk products at concentrations below 0.16 mg/kg.
- Lincomycin is a drug with a long history of use.
- A comparison of the microbiological assay and GC–MS with pig liver samples showed that the two assays give similar results, with a correlation coefficient greater than 0.98. The methods appear to be suitable for monitoring lincomycin at concentrations within the recommended MRLs.

The Committee recommended MRLs for lincomycin in pigs of 100 μg/kg for muscle and fat, 500 μg/kg for liver and 1500 μg/kg for kidney, expressed as parent drug. The Committee also recommended the same MRLs for lincomycin in the edible tissues of cattle, sheep and
chickens, but made them temporary, in accordance with its policy on
the evaluation of veterinary drugs with a long history of use (Annex
1, reference 104). The Committee also recommended an MRL of
150μg/kg for cows’ milk, expressed as parent drug.

The MRLs recommended above would result in a theoretical maxi-
mum daily intake of 385μg, based on a daily food intake of 300g of
muscle, 100g of liver, 50g each of kidney and fat, and 1.5 kg of milk.

The Committee was unable to recommend an MRL for chickens’ eggs.

The Committee required the following information for evaluation in
2002:

• Data from residue-depletion studies in cattle, sheep and chickens
  which show that lincomycin is the major microbiologically active
  residue in the edible tissues.

• Data from residue-depletion studies showing that lincomycin is the
  major microbiologically active residue in chickens’ eggs.

• The results of a residue-depletion study in which the GC-MS
  method is used to analyse residues in chickens’ eggs.

3.2.3 Oxytetracycline

Oxytetracycline was last evaluated by the Committee at its fiftieth
meeting (Annex 1, reference 134), when it established an ADI of
0–30μg/kg of body weight for oxytetracycline, tetracycline and
chlortetracycline, alone or in combination. At that meeting, it rec-
ommended MRLs of 200μg/kg for muscle, 600μg/kg for liver and
1200μg/kg for kidney, expressed as parent drug, alone or in combi-
nation with chlortetracycline and tetracycline, in cattle, pigs, sheep
and poultry. The Committee also recommended MRLs of 100μg/kg
for milk in cattle and sheep, and 400μg/kg for poultry eggs, expressed
as parent drug, alone or in combination with chlortetracycline and
tetracycline, and 200μg/kg for muscle in giant tiger prawns (Penaeus
monodon) and fish, expressed as parent drug alone. The MRL for
fish muscle was designated as temporary, pending evaluation of the
pattern of use of oxytetracycline in aquaculture.

The requested data were not submitted for consideration at the
present meeting; however, the Committee decided to extend the tem-
porary MRL for fish muscle until 2002, pending the results of a
residue-depletion study and information on the validation of the
analytical method for fish.

3.2.4 Tilmicosin

Tilmicosin was previously evaluated by the Committee at its forty-
seventh meeting (Annex 1, reference 125), when it established an
ADI of 0–40 µg/kg of body weight and recommended MRLs of 100 µg/kg for muscle and fat, 1000 µg/kg for liver and 300 µg/kg for kidney in both cattle and sheep, as well as 50 µg/kg for ewes' milk, expressed as parent drug. The recommended MRL for ewes' milk was designated as temporary, pending the results of a study with radiolabelled drug in lactating ewes, to determine the relationship between total residues and the parent drug in milk. The Committee also recommended MRLs of 100 µg/kg for muscle and fat, 1500 µg/kg for liver and 1000 µg/kg for kidney, expressed as parent drug, in pigs.

The requested information was not submitted for consideration at the present meeting. The Committee therefore decided not to extend the temporary MRL for ewes' milk. The MRLs for muscle, liver, kidney and fat of cattle, pigs and sheep were retained.

3.3 **Insecticides**

3.3.1 **Cyhalothrin**

Cyhalothrin is a type II pyrethroid insecticide and acaricide. Technical-grade cyhalothrin (about 98% pure), which was the material used in most of the toxicological studies, consists mainly of four of the possible 16 isomers. These four isomers comprise two pairs of enantiomers, A and B, in a ratio of 60:40. Within each pair, the enantiomers are present in equal amounts. A related product, λ-cyhalothrin, contains only the B pair of enantiomers.

Cyhalothrin is used predominantly on cattle and sheep, and to a lesser extent on pigs and goats, for the control of a broad range of ectoparasites, including flies, lice and ticks. Cyhalothrin is applied topically as a pour-on formulation to cattle at a dose of up to 60 ml (1.2 g) for ticks and 10 ml (0.2 g) for lice or flies, and to sheep and pigs at a dose of 5 ml (0.1 g) for all applications. Cyhalothrin is also available as a 20% (w/v) emulsifiable concentrate for use as a spray or a dip, prepared by dilution to 0.002–0.2%. It is applied at a dose of 0.1–4 litre per animal. In general, the more dilute the spray or dip, the greater the volume applied.

The Committee has not previously evaluated cyhalothrin. It was evaluated toxicologically by the 1984 Joint FAO/WHO Meeting on Pesticide Residues (8), when an ADI of 0–20 µg/kg of body weight was established. Residues of cyhalothrin were evaluated by the 1984, 1986 and 1988 Joint FAO/WHO Meetings on Pesticide Residues (8–10), which recommended MRLs for cabbage, cottonseed, cottonseed oil (crude and edible), pome fruits and potato, expressed as the sum of the cyhalothrin isomers.
Toxicological data

The Committee considered the results of studies on the pharmacokinetics, metabolism, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity, neurotoxicity and neurobehavioural effects of cyhalothrin and effects in humans exposed to the drug. Although some of the studies were not fully compliant with GLP, all of the pivotal studies were carried out according to appropriate standards for study protocol and conduct.

Oral doses of cyhalothrin were readily but incompletely absorbed by the experimental species studied (rats and dogs) and the subsequent metabolism was similar, involving initial cleavage of the molecule at the ester bond, presumably resulting in detoxification. The metabolites were rapidly excreted, some as conjugates, whereas small amounts of unchanged cyhalothrin persisted as residues in fatty tissues. The results were similar to those in food-producing animals.

Serum and urine from exposed workers contained the metabolites 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid, 3-phenoxycarboxylic acid and 3-(4'-hydroxyphenoxo)-benzoic acid. These metabolites were products of cleavage of the cyhalothrin molecule at the ester bond, and their presence suggests that the initial metabolism of this compound in humans is similar to that in the animal species that have been investigated.

No toxicological studies on metabolites of cyhalothrin were available, but the Committee considered it likely that the metabolites would be less toxic than cyhalothrin, as none contains an intact pyrethroid structure.

The acute toxicity of cyhalothrin is characterized by effects on the nervous system, principally the central nervous system. The signs of toxicity are typical of type II pyrethroids and comprise writhing, salivation, exaggerated jaw opening, increasing extensor tone in the hind legs causing a rolling gait, poor coordination progressing to coarse tremor, tonic seizures and apnoea. The LD$_{50}$ values after oral administration depended on the vehicle used, but varied from 37 to 62 mg/kg of body weight in mice and from 51 to 240 mg/kg of body weight in rats. Higher values were found in other species or after administration by other routes.

In mice that received a dose of 0, 5, 10, 20, 40 or 80 mg/kg of body weight per day orally for 5 days, ataxia and convulsions were seen in those given the two highest doses. The dose of 20 mg/kg of body weight per day caused body-weight loss, ataxia and roughness of the coat. At 5 mg/kg of body weight per day, the only adverse effect seen was a rough coat.
In a 28-day study in mice, cyhalothrin was added to the diet at a concentration of 0, 5, 25, 100, 500 or 2000 mg/kg of feed. Atrophy of the red pulp of the spleen and an increased mortality rate were seen at the highest dose. Adaptive liver changes, including enlarged liver, hypertrophy of the centrilobular hepatocytes, increased activity of aminopyrine N-demethylase, and proliferation of the smooth endoplasmic reticulum were seen at concentrations of 100 mg/kg of feed and above; lowered lymphocyte counts were also seen at these doses. At 25 mg/kg of feed, the only effect seen was piloerection. The NOEL was 5 mg/kg of feed, equal to 0.65 mg/kg of body weight per day, on the basis of piloerection at higher doses.

The main effects in five toxicity studies lasting 10–90 days in rats were adaptive changes in the liver similar to those seen in mice. In a 28-day range-finding study in which rats were fed diets containing cyhalothrin at a concentration of 0, 5, 10, 20 or 250 mg/kg of feed, only the highest concentration caused adverse effects. These effects were characterized by hepatocellular hypertrophy, increased activity of aminopyrine N-demethylase in the liver, and proliferation of hepatic smooth endoplasmic reticulum. The NOEL was 20 mg/kg of feed, equivalent to 2 mg/kg of body weight per day. In a 28-day study in rats given 0, 1, 5, 10, 20 or 250 mg/kg of feed, increased activity of aminopyrine N-demethylase and proliferation of smooth endoplasmic reticulum in the liver were seen at the highest dose only. No such effect was seen in the liver at doses equivalent to 2 mg/kg of body weight per day or less. Females given 10 mg/kg of feed (equivalent to 1 mg/kg of body weight per day) or more had decreased body-weight gain. The NOEL was 5 mg/kg of feed, equivalent to 0.5 mg/kg of body weight per day, which is lower than the levels found in other short-term studies in rats. A third 28-day study in rats was inadequately reported, but showed that administration of cyhalothrin at 20 mg/kg of feed (equivalent to 2 mg/kg of body weight per day) caused proliferation of hepatic smooth endoplasmic reticulum; a NOEL was not identified in this study.

A 90-day toxicity study was performed in which rats were given cyhalothrin at 0, 10, 50 or 250 mg/kg of feed. Males given the two highest doses showed increased activity of aminopyrine N-demethylase and proliferation of smooth endoplasmic reticulum in the liver. Females given the highest dose showed increased activity of hepatic aminopyrine N-demethylase activity. The NOEL was 10 mg/kg of feed, equal to 0.56 mg/kg of body weight per day.

Groups of dogs received cyhalothrin at a dose of 0, 2.5, 10 or 30 mg/kg of body weight per day for 4 weeks or a dose of 0, 1.0, 2.5 or 10 mg/kg of body weight per day for 26 weeks. In the 4-week study, the two
highest doses caused an increased incidence of vomiting, and the highest dose caused body-weight loss, unsteady gait and increased serum activities of alanine and aspartate aminotransferases. Muscular trembling was seen in dogs in all groups, including the controls, in the 4-week study. In the 26-week study, doses of 10 mg/kg of body weight per day or more caused clinical signs of toxicity, including vomiting, salivation, lack of coordination, unsteadiness, collapse, muscular spasms and convulsions. As treatment at all doses in both studies increased the prevalence of liquid faeces, a NOEL was not identified. The Committee considered it possible that the liquid faeces were a consequence of the neurological effects of cyhalothrin. The lowest-observed-effect level (LOEL) was 1.0 mg/kg of body weight per day.

In a long-term study of toxicity and carcinogenicity in mice, cyhalothrin was given in the diet at a concentration of 0, 20, 100 or 500 mg/kg of feed for 104 weeks. An increased incidence of mammary adenocarcinomas was seen in treated females at the two highest doses. The highest incidence (14%) was only slightly greater than the upper limit of the range in historical controls (2–12%). The Committee could not exclude the possibility that the adenocarcinomas seen in the groups given 100 or 500 mg/kg of feed were caused by cyhalothrin. Clinical signs of toxicity (piloerection and hunched posture) and increased serum activities of aspartate and alanine aminotransferases were seen at these doses. The NOEL was 20 mg/kg of feed, equal to 1.9 mg/kg of body weight per day, on the basis of these effects.

In a long-term study of toxicity and carcinogenicity in rats, cyhalothrin was given in the diet at a concentration of 0, 10, 50 or 250 mg/kg of feed for 104 weeks. There was no treatment-related increase in the incidence of any type of tumour. Adverse effects found at the highest dose included decreased body weight, altered blood biochemistry and increased relative weight of the liver in both sexes and of the adrenals in females. The NOEL was 50 mg/kg of feed, equal to 2.3 mg/kg of body weight per day, on the basis of these effects.

Cyhalothrin was not genotoxic in a range of studies, including a test for reverse mutation in Salmonella typhimurium, a test for cytogenetic effects in the bone marrow of rats treated in vivo, a test for dominant lethal mutation in mice, and an assay of cell transformation in vitro. The Committee concluded that cyhalothrin is not genotoxic. Furthermore, the Committee considered it likely that the mammary adenocarcinomas found in the long-term toxicity/carcinogenicity study in mice were due to a non-genotoxic mechanism.
A three-generation study of reproductive toxicity was performed in rats given cyhalothrin in the diet at a concentration of 0, 10, 30 or 100 mg/kg of feed. Adverse effects, including reduced parental body-weight gain and reduced litter size, were found only at the highest dose. The NOEL for these effects was 30 mg/kg of feed, equal to 1.7 mg/kg of body weight per day.

In a study of developmental toxicity, rats received a dose of 0, 5, 10 or 15 mg/kg of body weight per day by gavage on days 6–15 of gestation. Maternal toxicity, characterized by body-weight loss and poor coordination, was seen at the highest dose. Embryotoxicity also occurred at this dose. Abnormalities were seen in 5 of 17 fetuses in one litter from a dam at 10 mg/kg of body weight per day, but the effect was considered not to be due to treatment with cyhalothrin as no fetotoxicity was seen at the higher dose. The NOEL for maternal toxicity was 10 mg/kg of body weight per day, and that for developmental toxicity was 15 mg/kg of body weight per day, the highest dose tested.

Two studies of developmental toxicity in rats treated by dermal administration provided some evidence that cyhalothrin can delay fetal development at doses lower than those administered orally in the study described above. However, the Committee considered that oral administration is a more relevant route and that the NOEL in the study in which this route was used was the appropriate one for evaluating developmental toxicity in rats.

A study of developmental toxicity was conducted in rabbits, which received cyhalothrin at a dose of 0, 3, 10 or 30 mg/kg of body weight per day by gavage. The highest dose caused initial body-weight loss in the does, which was followed by reduced body-weight gain. There was no significant effect on development at any dose. The NOEL for maternal toxicity was 10 mg/kg of body weight per day, and that for developmental toxicity was 30 mg/kg of body weight per day, the highest dose tested.

Single oral doses of up to 10 g/kg of body weight did not induce clinical or histopathological signs of neurotoxicity in hens.

Various studies of neurobehavioural effects induced by cyhalothrin have been conducted in rats, including a range-finding test of performance on an inclined plane, a test for acute auditory startle response and tests of inhibitory avoidance. In the inclined plane test, rats were given a single oral dose of 0, 15, 25, 30, 40, 50, 60, 75, 100 or 200 mg/kg of body weight. All of these doses caused soft faeces in at least some of the rats, and doses of 25 mg/kg of body weight or more caused clinical signs of neurotoxicity. The results were, however,
variable, and no conclusion could be reached about the neurotoxic effects of the compound.

In the test for acute auditory startle response, in which a single oral dose of 0, 5, 15 or 75 mg/kg of body weight was given, the highest dose resulted in reduced body-weight gain, transient clinical signs of toxicity and a reduced amplitude of the mean response in animals of both sexes. Statistically significant, but not dose-related, reductions in amplitude of the mean startle response were also seen at 5 and 15 mg/kg of body weight in females only. The Committee was aware that a biphasic auditory startle response had been reported with some other type II pyrethroids, with a reduced response at high doses and an increased response at lower doses. A NOEL for the startle response was not identified in this study.

In the inhibitory avoidance tests, rats were exposed either in utero (dams were given 200 mg/l in drinking-water) or during lactation (dams were given 1 ml of a 0.018% solution dermally); however, the doses received by the animals could not be estimated reliably. The rats exposed in utero showed a decreased motivational response when tested as adults, but no effects were seen on inhibitory avoidance behaviour or locomotor frequency. Animals exposed during lactation had a shorter latency in learning avoidance behaviour. There was no NOEL in either study, as adverse effects were seen at the only dose tested in each study and the doses received by the animals were not known precisely.

Observations and case reports in humans provided little information relevant to the establishment of an ADI for cyhalothrin. In most instances, no information on doses was available, the route of exposure was dermal, and some studies were of exposure to λ-cyhalothrin rather than cyhalothrin.

The Committee established a temporary ADI of 0–2 μg/kg of body weight for cyhalothrin by applying a 500-fold safety factor to the LOEL of 1.0 mg/kg of body weight per day for induction of liquid faeces in dogs in the 26-week study. The Committee considered it possible that the liquid faeces were a consequence of the neurological effects of cyhalothrin. The Committee applied the 500-fold safety factor to account for the absence of a NOEL for liquid faeces in dogs and for the absence of a NOEL for neurobehavioural effects. The ADI was made temporary because cyhalothrin belongs to a class of substances that are characterized by their toxicity to the central nervous system, and therefore neurobehavioural effects may be the most sensitive indicator of the toxicity of this compound. There was an adequate margin of safety between the ADI and the NOELs identified.
in other studies on cyhalothrin, including the NOEL of 0.65 mg/kg of body weight per day for piloerection in the 28-day study in mice.

The results of studies appropriate for identifying a NOEL for neurobehavioural effects in laboratory animals are required for evaluation in 2002.

The Committee noted that the NOEL for toxicological effects other than the neurotoxicity related to the pyrethroid structure was 2.3 mg/kg of body weight per day for adaptive changes in the livers of rats in the long-term toxicity/carcinogenicity study. This suggests that the toxicity of the metabolites (none of which has a pyrethroid structure) is no greater than 50% of that of the parent drug.

Pharmacokinetic data
The results of studies in laboratory and food-producing animals show consistently that more than 90% of a dose of cyhalothrin is eliminated rapidly in the urine and faeces. The tissue in which residues are found primarily is fat, and the residues occur as the isomers of cyhalothrin, although in proportions different from those in formulated products. Cyhalothrin is eliminated in milk as the isomers found in the original product.

Cattle. In a study conducted according to GLP, $[^{14}C]$cyhalothrin was given orally twice daily at a dose of 1 mg/kg of body weight for 7 days to two cows. The first cow received $[^{14}C]$cyhalothrin labelled in the C1-position of the cyclopropane ring (cyclopropyl label), while the second received $[^{14}C]$cyhalothrin labelled at the $\alpha$-carbon of the benzyl group (benzyl label). When the cows were killed 16 h after the final treatment, most of the radiolabel had been eliminated in faeces (49%) and urine (27%). The major metabolites identified in urine, kidney and liver from the cow given cyclopropyl-labelled cyhalothrin were 16-(1RS)-cis-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylic acid (CPA) and its conjugates, while the glutamic acid derivative of 3-phenoxycbenzoic acid was the major urinary metabolite identified after administration of benzyl-labelled cyhalothrin. The parent compound was the major component of the total residues in faeces and in muscle, fat and milk; it accounted for 10% or more of the total residues in kidney, but less than 5% of those in liver. Confirmation was provided by studies conducted with $[^{14}C]$-cyhalothrin, which contains only the B-enantiomer pair of the isomeric mixture.

Residue data
All of the studies summarized in this section were conducted in compliance with GLP, and the cyhalothrin residues in the studies of unlabelled compound were measured as the sum of the isomers.
Cattle. In a study in which one cow received $[^{14}\text{C}-1\text{-cyclopropyl}]$cyhalothrin and a second cow received the benzyl-labelled compound orally twice daily at 1 mg/kg of body weight for 7 days, the concentrations of total residues were higher in the tissues of the animal treated with the cyclopropyl-labelled drug. This indicates greater retention of the CPA fragment and its conjugates than of the fragments from the remainder of the cyhalothrin molecule after cleavage at the ester bond. The total concentrations of radiolabel in tissues were highest in perirenal fat (2.7 mg/kg), followed by subcutaneous fat (1.6 mg/kg), liver (1.3 mg/kg), kidney (0.60 mg/kg) and muscle (0.19 mg/kg). In milk, the highest concentration measured was 0.59 mg/kg, and this was associated with the cream fraction. The usual 60:40 ratio of A:B isomers in the formulated product was essentially reversed in perirenal fat and in milk, suggesting that the B isomers are more persistent than the A isomers. Confirmatory data on the distribution of cyhalothrin in tissues was provided by studies conducted with $[^{14}\text{C}]\text{L}$-cyhalothrin, which contains the more toxic B-enantiomer pair.

Groups of five calves weighing 105–165 kg received cyhalothrin from two tapes attached to an ear tag and from seven spray treatments (at a dose equivalent to 2 mg/kg of body weight) at 14-day intervals. The tag tapes were an experimental product that is not currently recommended for use, and the recommended time between spray applications is 2–4 weeks. The animals were slaughtered in groups of five at 0, 3, 7 and 14 days after the last spray treatment. The highest concentrations of residues were found in perirenal fat and decreased from 0.32 mg/kg (standard deviation, 0.13 mg/kg) immediately after the end of treatment to 0.16 mg/kg (standard deviation, 0.03 mg/kg) 14 days after treatment. The concentrations of residues were consistently 10–25% lower in subcutaneous fat than in perirenal fat. Immediately after the end of treatment, the concentrations were 0.005 mg/kg in liver and muscle and 0.010 mg/kg in kidney. The treatment schedule was considered to represent the most extreme use of cyhalothrin as the spray product.

Four applications of a pour-on formulation of cyhalothrin were applied topically at weekly intervals to cattle weighing 250–350 kg at a dose equivalent to 2 mg/kg of body weight. Although more frequent application may be required for treatment of ticks, the recommended treatment interval for the pour-on preparation is 4–8 weeks. The highest concentration of residues in animals slaughtered 7 h after the final application was found in perirenal fat (0.91 mg/kg; standard deviation, 0.37 mg/kg). In animals slaughtered 14 days after the final treatment, the concentrations were 0.91 mg/kg (standard deviation,
0.55 mg/kg) in perirenal fat and 0.35 mg/kg (standard deviation, 0.17 mg/kg) in subcutaneous fat. The concentrations in all liver samples were at or below the limit of quantification of the analytical method (0.01 mg/kg). Samples of other tissues were not analysed in this study.

After a single pour-on application of cyhalothrin solution to five dairy cows at a dose equivalent to 0.4 mg/kg of body weight, the highest concentration of residues found in milk was 0.04 mg/kg (standard deviation, 0.01 mg/kg) at the fifth milking (day 3); the value had decreased to less than 0.01 mg/kg by day 7.

Five dairy cows received seven spray treatments with cyhalothrin at 14-day intervals, each at a dose of about 2 mg/kg of body weight. Most of the 150 milk samples collected and analysed during treatment contained concentrations of residues in excess of 0.005 mg/kg, exceeding 0.01 mg/kg in only 15 samples. The highest concentrations were found in milk samples taken 2–3 days after each application, and the highest mean concentration was 0.012 mg/kg (standard deviation, 0.005 mg/kg) in milk collected 2 days after the third spraying. In a subsequent study, five cows received four repeated spray treatments at a dose of approximately 2.9 mg/kg of body weight with a 7-day interval between treatments. The concentration of residues in milk typically reached a maximum 3–4 days after spraying, with a maximum mean concentration of 0.013 mg/kg (standard deviation, 0.004 mg/kg) at the sixth milking, 3 days after the third spray application. In animals slaughtered 16 h after the final spraying, the concentrations of residues in tissues were 0.10 mg/kg (standard deviation, 0.03 mg/kg) in perirenal fat, 0.16 mg/kg (standard deviation, 0.22 mg/kg) in subcutaneous fat, and below 0.01 mg/kg in liver, kidney and muscle.

Four dairy cows weighing 480–535 kg received four applications at 7-day intervals of a pour-on formulation of cyhalothrin at a dose equivalent to 1.2 mg/kg of body weight. Four cows in a second group each received a single application of 3.6 mg/kg of body weight, followed by three weekly applications of 2.4 mg/kg of body weight. After the dose of 1.2 mg/kg of body weight, the highest concentrations of residues were found at the third to fifth milkings after the initial application, with a maximum of 0.18 mg/kg (standard deviation, 0.11 mg/kg). The mean concentrations were below 0.005 mg/kg by the time of the seventh milking after the final application. In the cows that received the dose of 3.6 mg/kg of body weight, the highest concentration of residues found in milk was 0.47 mg/kg on day 3 after the first application, while the highest concentration found after the first application of 2.4 mg/kg of body weight was 0.30 mg/kg at the third milking.
of one cow. The highest concentrations were found in milk from the third to seventh milkings after the initial treatment, and the concentration was not consistently below 0.005 mg/kg until 16 milkings after the final treatment. The limit of quantification of the analytical method was 0.01 mg/kg. The recommended interval between applications of the pour-on product in cattle is 4–8 weeks.

**Pigs.** Groups of four pigs weighing 39–50 kg received a single application of 5 ml of a pour-on formulation of 2% cyhalothrin (equivalent to 3 mg/kg of body weight) and were killed 3, 7 or 14 days later. The concentrations of residues were highest in skin at the site of application, decreasing from 0.82 mg/kg (standard deviation, 0.44 mg/kg) at day 3 to 0.33 mg/kg (standard deviation, 0.13 mg/kg) at day 14. In skin remote from the site of application, the concentrations decreased from 0.23 mg/kg (standard deviation, 0.04 mg/kg) at day 3 to 0.09 mg/kg (standard deviation, 0.08 mg/kg) at day 14. The concentrations in abdominal and subcutaneous fat were 0.08 mg/kg (standard deviation, 0.10 mg/kg) and 0.08 mg/kg (standard deviation, 0.05 mg/kg) at day 3 and 0.04 mg/kg (standard deviation, 0.01 mg/kg) and below 0.01 mg/kg at day 14, respectively. No residues of cyhalothrin were detected in liver, kidney or muscle at day 3.

**Sheep.** Four groups of three sheep weighing 28–36 kg received three 5-ml applications of a 2% pour-on formulation of cyhalothrin (equivalent to 2.2 mg/kg of body weight) from a syringe directly onto the skin at 14-day intervals. The groups were killed at 16h and 3, 7 and 14 days after the final treatment. The concentrations of residues were similar in subcutaneous and perirenal fat (0.03–0.13 mg/kg) taken at 16h and 3 and 7 days. No residues were detected in subcutaneous fat (limit of detection, 0.01 mg/kg) at day 14, but the concentration in perirenal fat at this time was 0.04–0.10 mg/kg. No residues were detected in liver (limit of detection, 0.05 mg/kg) or in kidney or muscle (limit of detection, 0.01 mg/kg) samples at any time after treatment.

**Analytical methods**

The method used to measure residue depletion is based on gas chromatography with detection by electron capture (GC–ECD). Validation was provided for the analysis of cyhalothrin residues in edible tissues from cattle, pigs and sheep and in cows' milk. The method involves extraction of the sample, followed by solvent partitioning and solid-phase extraction for purification of the extracts. The choice of cartridge depends on the sample matrix and the detection system. The analytical recovery was 74–100%, depending on the sample matrix. The limit of detection for the assay is 0.001–0.005 mg/kg, and the limit of quantification is 0.01 mg/kg for all tissues (except sheep liver,
0.05 mg/kg) and milk. The isomers appear as a single chromatographic peak in this method. No interference was found from the matrix or from the related synthetic pyrethroids deltamethrin, cypermethrin, permethrin, fenvalerate, cyfluthrin or flumethrin. An additional method was provided in which gas chromatography is used, with detection by mass spectrometry using chemical ionization in the negative ion mode. The limits of detection and of quantification are somewhat higher than those of the GC--ECD method, and the isomers are partially resolved in the chromatographic separation. The GC--ECD method has been used successfully in several laboratories since the 1990s and in a number of trials.

**Maximum Residue Limits**

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

- A temporary ADI of 0–2 μg/kg of body weight was established by the Committee on the basis of a toxicological end-point, which would permit a maximum ADI of 120 μg for a 60-kg person.
- The sum of the isomers of cyhalothrin is the appropriate marker residue, as previously established by the 1984 Joint FAO/WHO Meeting on Pesticide Residues (8).
- Cyhalothrin isomers account for less than 5% of the total residues in cattle liver and 10% or more of those in kidney. The metabolites were considered to be half as toxic as the parent compound. The marker residue accounted for about 6% of the total residues in liver and 20% of those in kidney.
- The residues found in muscle, fat and milk consisted of the parent compound.
- Weekly application of the pour-on formulation to cattle and the use of more than 10 ml of this product result in much higher concentrations of residues in fat and milk than are found with less frequent application of smaller volumes. While such usage may be within the range of the recommended applications of cyhalothrin, it was considered to be an unsuitable basis for establishing MRLs as it represents extreme usage. Use of the pour-on product can result in residue concentrations in excess of the MRL for milk.
- The maximum intakes allowed by the 1988 Joint FAO/WHO Meeting on Pesticide Residues (10) for horticultural use account for 10% or less of the temporary ADI established by the Committee.
- A suitable analytical method is available for analysis of cyhalothrin residues in edible tissues and milk.
- The recommended MRLs for liver, kidney and muscle are based on twice the limit of quantification of the analytical method as validated for tissues and harmonized for cattle, pigs and sheep.
Table 2
Theoretical maximum daily intake of cyhalothrin residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)a</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intakeb (µg cyhalothrin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>20</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Liver</td>
<td>20</td>
<td>333c</td>
<td>33</td>
</tr>
<tr>
<td>Kidney</td>
<td>20</td>
<td>100d</td>
<td>5</td>
</tr>
<tr>
<td>Fat</td>
<td>400</td>
<td>400</td>
<td>20</td>
</tr>
<tr>
<td>Milk</td>
<td>30</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>109</td>
</tr>
</tbody>
</table>

a Expressed as parent drug.
b Based on a daily intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 kg of milk.
c The marker residue accounted for 6% of the total residues in liver.
d The marker residue accounted for 20% of the total residues in kidney.

- The recommended MRLs for fat are based on the upper confidence limit of the highest mean concentration of residues as determined in residue-depletion studies in which the product was used in accordance with good practice in the use of veterinary drugs. The MRL recommended for milk is based on the upper confidence limit of the highest mean concentration of residues, as determined in residue-depletion studies in which treatment with the spray formulation was given in accordance with good practice in the use of veterinary drugs.

The Committee recommended temporary MRLs in cattle, pigs and sheep of 20 µg/kg for muscle, liver and kidney and 400 µg/kg for fat, expressed as parent drug. The Committee also recommended a temporary MRL of 30 µg/kg for cows’ milk.

From these MRLs, the theoretical maximum daily intake of cyhalothrin residues due to veterinary use would be 109 µg (Table 2). Since the maximum ADI is 120 µg, this gives a margin of safety for the possible ingestion of residues from other uses.

Data on the validation of the analytical method for sheep liver, to confirm the limit of quantification of 10 µg/kg, are required for evaluation in 2002.

3.3.2 Cypermethrin

Cypermethrin was previously evaluated by the Committee at its forty-seventh meeting (Annex 1, reference 125), when it established an ADI of 0-50 µg/kg of body weight and recommended temporary MRLs of 200 µg/kg for muscle, liver and kidney and 1000 µg/kg for fat, expressed as parent drug, in cattle, sheep and chickens. It also
recommended temporary MRLs of 50\(\mu\)g/kg for milk (whole) and 100\(\mu\)g/kg for eggs, expressed as parent drug, in cattle and chickens, respectively.

At its forty-seventh meeting, the Committee required the following information for evaluation in 2000:

1. The results of residue-depletion studies with radiolabelled cypermethrin that extend beyond the recommended withdrawal times for the drug in its topical formulation. The depletion of the total residues and of the parent drug should be determined.
2. Evidence that interconversion of the isomeric forms does not occur during metabolism in the target species.
3. Further information on the validation of the analytical methods, particularly data on the derivation of the limits of detection and quantification.

At the present meeting, the Committee noted that no information had been provided to answer the first and second requests and that there was no indication that the sponsors would provide this information in the near future.

A study in sheep treated orally with an isomeric mixture of radiolabelled cypermethrin was available. This study did not address topical administration of the drug, and the ratio of cis:trans isomers (80:20) was different from that of the isomeric mixture of cis:trans cypermethrin (45:55) which had been evaluated at the forty-seventh meeting.

In response to the third request, a suitable analytical method was provided for quantifying the total residues resulting from the use of the cypermethrin formulation containing an 80:20 mixture of the cis- and trans-isomers. Since the method uses gas chromatography, in which all the possible isomers co-elute, it can be used to quantify total residues resulting from the use of cypermethrin containing any ratio of cis- and trans-isomers.

Since the Committee did not receive the required information and there was no indication that it would be provided in the future, the temporary MRLs recommended at the forty-seventh meeting were not extended.

The Committee also noted that no information was available for a toxicological evaluation of the 80:20 cis:trans isomeric mixture of cypermethrin.

3.3.3 \(\alpha\)-Cypermethrin

\(\alpha\)-Cypermethrin was previously evaluated by the Committee at its forty-seventh meeting (Annex 1, reference I25), when it established
an ADI of 0–20μg/kg of body weight and recommended temporary MRLs of 100μg/kg for muscle, liver and kidney and 500μg/kg for fat, expressed as parent drug, in cattle, sheep and chickens. It also recommended temporary MRLs of 25μg/kg for whole milk and 50μg/kg for eggs, expressed as parent drug, in cattle and chickens, respectively.

At its forty-seventh meeting, the Committee required the following information for evaluation in 2000:

1. The results of residue-depletion studies with radiolabelled α-cypermethrin in sheep and chickens that extend beyond the recommended withdrawal times for the drug in its topical formulation. The depletion of the total residues and of the parent drug should be determined.

2. The residue-depletion study with radiolabelled α-cypermethrin in cattle that was submitted should be reassessed to determine the depletion of the total residues and of the parent drug.

3. Evidence that the interconversion of isomeric forms of α-cypermethrin does not occur during metabolism in the target species.

4. Further information on the validation of the analytical methods, particularly data on the derivation of the limits of detection and quantification.

Since the Committee did not receive the required information and there was no indication that it would be provided in the future, the temporary MRLs recommended at the forty-seventh meeting were not extended.

3.3.4 Dicyclanil

Dicyclanil has not previously been evaluated by the Committee. It is a pyrimidine-derived insect growth regulator used for the topical treatment of sheep to prevent larval infestation by the blowfly (Lucilia cuprina). It is applied as a pour-on formulation containing 5% (w/v) of the drug.

At the present meeting, data were provided on the use of dicyclanil applied as a pour-on formulation to sheep at a maximum dose of 0.1g/kg of body weight.

Toxicological data

The Committee considered the results of studies on the pharmacokinetics, metabolism, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity and pharmacology of dicyclanil. All of the pivotal studies were carried out according to appropriate standards for study protocol and conduct.
After repeated oral administration of radiolabelled dicyclanil to rats, the radiolabel was rapidly and almost completely absorbed and distributed to the major organs and tissues. Elimination was rapid (93% or more of the total dose within 24h) and was virtually complete within 3 days. The major route of elimination was the urine (79–83% of the total dose within 24h), while the faecal route was of minor importance (6–12% of the total dose within 24h). Biotransformation in the rat involves oxidative opening of the cyclopropyl ring at various positions, followed by further oxidation and cleavage of the cyclopropyl-N bond (i.e. dealkylation). The pathways by which dicyclanil is metabolized in sheep treated topically are essentially the same as those in rats.

After oral administration of dicyclanil to rats, the LD$_{50}$ values were 560mg/kg of body weight in males and 500mg/kg of body weight in females. Dicyclanil is moderately hazardous when given as a single oral dose.

In a 3-month study of toxicity, rats received dicyclanil in the diet at a concentration of 0, 5, 25, 125 or 500mg/kg of feed. Males showed decreased food consumption and body-weight gain and slightly decreased plasma glucose concentrations at 125 and 500mg/kg of feed; the relative weights of the kidneys, brain and testes were increased in the highest-dose group. Females in the highest-dose group showed decreased food consumption, body-weight gain and plasma glucose concentrations and increased relative weights of the liver and brain; plasma glucose concentrations were also reduced at 125mg/kg of feed. The NOEL was 25mg/kg of feed, equal to 1.6mg/kg of body weight per day, on the basis of the reduction in body-weight gain.

In a 3-month study of toxicity, dogs received dicyclanil in the diet at a concentration of 0, 20, 100, 500 or 1500mg/kg of feed. Clinical signs of toxicity, including signs of neurotoxicity, decreased food consumption and body-weight gain, and changes in clinical chemistry and erythrocyte parameters were observed mainly at the highest concentration. Plasma cholesterol and phospholipid concentrations were increased in animals at concentrations of 100mg/kg of feed and above. The weights of the spleen (males and females) and thymus and testes (males only) were decreased at 1500mg/kg of feed. Atrophy of the spleen in males and females and atrophy of the thymus, mesenteric lymph node, testes and prostate in males were observed at concentrations of 100mg/kg of feed and above. The weights of the liver, adrenals and kidneys were increased in animals in the highest-dose group; in females, the liver weights were also increased at lower
doses. Inflammatory changes were seen in the urinary bladder of females at concentrations of 100mg/kg of feed and above and in the liver of males and females at 1500mg/kg of feed. Hepatocyte oedema was observed in females in all treatment groups, but hepatocellular damage was not seen in any of the treatment groups. As the Committee considered hepatocyte oedema to be without toxicological significance in the absence of hepatocellular damage, the NOEL was 20mg/kg of feed, equal to 0.61mg/kg of body weight per day, on the basis of increased plasma cholesterol concentrations and histopathological alterations of the prostate and urinary bladder.

In a 1-year study of toxicity, groups of four male and four female dogs received dicyclanil in the diet at a concentration of 0, 5, 25, 150 or 750mg/kg of feed. Males showed slightly decreased plasma calcium concentrations and increased absolute and relative liver weights at 750mg/kg of feed. Plasma cholesterol concentrations were increased in males at 150 and 750mg/kg of feed, and this effect was not reversed after a 4-week recovery period. Females receiving the highest dose vomited and had slightly reduced food consumption and body-weight gain, slightly increased plasma cholesterol concentrations, increased absolute and relative liver weights, and decreased absolute and relative heart weights. Macroscopic and microscopic findings consisting of necrosis of the liver and tubular lesions in the kidneys were found in one male and one female in the highest-dose group that died prematurely; atrophy of the testes and prostate (in the male) and vascular thrombus (in the female) were also observed. These animals suffered from acute, severe liver failure, cardiovascular disturbances and stress due to weight loss. Comparable acute, severe liver toxicity was not observed in the 3-month study of toxicity in dogs given dicyclanil in the diet at concentrations of up to 1500mg/kg of feed. The findings in the two animals that died prematurely were therefore considered to be incidental. The NOEL was 25mg/kg of feed, equal to 0.71mg/kg of body weight per day, on the basis of increased plasma cholesterol concentrations in male dogs. This NOEL is supported by the NOEL in the 3-month toxicity study in dogs. It should be noted that the histopathological alterations observed in the 3-month study were not seen among the surviving animals in the 1-year study.

In a study of carcinogenicity, mice received diets containing dicyclanil at a concentration of 0, 10, 100, 500 or 1500mg/kg of feed for 18 months. The animals in the highest-dose group were killed during weeks 58–59 because of self-inflicted injuries and poor health. On the basis of significant reductions in body-weight gain, the concentrations of 500 and 1500mg/kg of feed in females and 1500mg/kg of feed in males were considered to exceed the maximum tolerated dose.
The liver was the main target organ in both male and female mice. The effects included pigmentation of the Kupffer cells (with haemosiderin) and hepatocellular necrosis in males at concentrations of 100mg/kg of feed and above, an increased incidence of hepatocellular adenomas in females at 500 and 1500mg/kg of feed, and an increased incidence of hepatocellular carcinomas in females at 1500mg/kg of feed. The Committee noted that these liver tumours were observed only at doses that exceeded the maximum tolerated dose and that there were signs of hepatocellular proliferation in these animals, which might have been involved in the hepatic carcinogenesis observed. Pigmentation of the olfactory epithelium (with oxidized lipofuscins) was observed in both sexes at 100 and 500mg/kg of feed; in males, this effect was accompanied by an increased incidence of inflammatory cell infiltration in the underlying Bowman glands. Males and females in the group treated at 500mg/kg of feed also showed pigmentation of the adrenal glands (with partly oxidized lipofuscins) and hypercellularity of the bone marrow. As the Committee considered the effects on the olfactory epithelium to be of no biological significance, the NOEL was 10mg/kg of feed, equal to 1.1mg/kg of body weight per day, on the basis of the effects on the liver.

In a 2-year study of carcinogenicity and toxicity, rats received diets containing dicyclanil at concentrations of 0, 5, 25, 125 or 500mg/kg of feed. Animals in the highest-dose group showed decreased food consumption and body-weight gain and increased relative weights of almost all organs. Treatment-related histopathological alterations were observed in the exocrine pancreas (hyperplasia) of males and in the liver (biliary cysts) of females at the highest dose, and in the olfactory epithelium (pigmentation resulting from accumulation of oxidized lipofuscins) of males at concentrations of 25mg/kg of feed and above and of females at concentrations of 125mg/kg of feed and above. Although the alterations in the olfactory epithelium represent enhancement of a naturally occurring age-related process, treatment had no effect on survival, behaviour or general well-being, and there were no other morphological changes in the olfactory mucosa. The Committee therefore concluded that the effect on the olfactory epithelium was of no biological significance. Dicyclanil did not affect the incidence of tumours. The NOEL was 125mg/kg of feed, equal to 22mg/kg of body weight per day, on the basis of changes in body weight and histopathological changes in the liver and pancreas.

Dicyclanil has been tested in vitro for its ability to induce reverse mutations in Salmonella typhimurium and Escherichia coli, gene mutations in Chinese hamster lung cells, chromosomal aberrations in Chinese hamster ovary cells and unscheduled DNA synthesis in pri-
mary rat hepatocytes. It has also been tested in vivo for its ability to induce micronuclei in bone-marrow cells of mice treated orally. The results of all of these tests were negative. On the basis of these data, the Committee concluded that dicyclanil is not genotoxic.

The Committee concluded that dicyclanil does not represent a carcinogenic risk for humans, as the liver tumours observed in female mice occurred in only one tissue of animals of one sex and one species and at levels that were above the maximum tolerated dose.

In a two-generation study of reproductive toxicity, with two litters per generation, rats were given dicyclanil in the diet at a concentration of 0, 5, 30, 200 or 500 mg/kg of feed. Treatment reduced the body-weight gain of the parental animals at 500 mg/kg of feed and, marginally, at 200 mg/kg of feed. Secondary to this effect, dicyclanil increased the relative weights of most organs in animals at 500 mg/kg of feed and of the brain (males and females) and kidneys and testes (males) at 200 mg/kg of feed. Reproductive parameters were not affected. The only effect of dicyclanil on pups was to reduce their weight gain from day 4 postpartum onwards. The NOEL for parental toxicity was 30 mg/kg of feed, equal to 2 mg/kg of body weight per day, on the basis of reduced body-weight gain. The NOEL for reproductive toxicity was 500 mg/kg of feed, equal to 24 mg/kg of body weight per day, the highest dose tested. The NOEL for toxicity to the pups was 200 mg/kg of feed, equal to 21 mg/kg of body weight per day, on the basis of reduced body-weight gain.

In a study of developmental toxicity in rats given dicyclanil at a dose of 0, 1, 5, 25 or 75 mg/kg of body weight per day orally on days 6–15 of gestation, the highest dose was toxic to the dams, as seen by reductions in body-weight gain, food consumption and the absolute weight of the gravid uterus. Marginal reductions in body-weight gain and food consumption were also observed at 25 mg/kg of body weight per day. The effects on the fetuses, observed only at the highest dose, were reduced weight, a slightly increased incidence of dilatation of the renal pelvis, and a number of mainly sternebral defects and variations due to poor or absent ossification. There was no evidence of teratogenicity. The NOEL for maternal toxicity was 5 mg/kg of body weight per day, on the basis of the reduction in body-weight gain. The NOEL for developmental toxicity was 25 mg/kg of body weight per day, on the basis of reduced fetal weight, increased dilatation of the renal pelvis, and increased skeletal anomalies and variations consistent with a slight delay in skeletal maturation.

In a study of developmental toxicity in rabbits given dicyclanil at a dose of 0, 1, 3, 10 or 30 mg/kg of body weight per day orally on days
7–19 of gestation, dams in the highest-dose group showed reduced food consumption and body-weight gain; reduced body-weight gain was also observed in dams at 10mg/kg of body weight per day. The fetuses of dams in the highest-dose group had lower body weights than controls and an increased incidence of skeletal variations indicative of a slight delay in ossification. There was no evidence of teratogenicity. The NOEL for maternal toxicity was 3mg/kg of body weight per day, on the basis of reduced body-weight gain. The NOEL for developmental toxicity was 10mg/kg of body weight per day, on the basis of reduced fetal weight and skeletal variations consistent with delayed ossification.

In pharmacological tests in vitro, dicyclanil had no effect on the skeletal neuromuscular junction at doses of up to 3mmol/l. At concentrations of 0.3mmol/l and higher, it had slightly antagonistic effects on smooth muscle contractions induced by agonists. In mice and rats given a single oral dose of 0, 1, 10, 50 or 100mg/kg of body weight, the highest dose affected general behaviour, locomotor activity, motor coordination, heart rate, and tidal and minute lung volume. Locomotor activity was also affected at 10mg/kg of body weight and, very slightly, at 1mg/kg of body weight. Treatment had no effect on body temperature, hypnotic potentiation, gastrointestinal motility, blood pressure, heart beat or respiratory rate.

The Committee established an ADI of 0–7μg/kg of body weight for dicyclanil, based on the NOEL of 0.71mg/kg of body weight per day for increased plasma cholesterol concentrations in the 1-year study of toxicity in dogs and a safety factor of 100.

**Pharmacokinetic and metabolism data**

*Rats.* Studies in rats showed that the major metabolites of dicyclanil in urine, faeces and tissues were parent dicyclanil, 2,4,6-triaminopyrimidine-5-carbonitrile (descyclopropyl dicyclanil), N-(4,6-diamino-5-cyanopyrimidin-2-yl)propionamide, 2-(4,6-diamino-5-cyanopyrimidin-2-ylamino)-3-hydroxypropionic acid, and 3-(4,6-diamino-5-cyanopyrimidin-2-ylamino)propionic acid. Thin-layer chromatography (TLC) and HPLC were used to separate 12 metabolite fractions from urine, faeces and tissues. The pattern did not vary by sex or dose. The major urinary metabolite, N-(4,6-diamino-5-cyanopyrimidin-2-yl)propionamide, represented 50% of the total dose.

*Sheep.* Three studies were available in which sheep were treated with a [14C]dicyclanil formulation typical of those used in current veterinary applications. In two studies, the pour-on formulation was used,
and this resulted in greater dermal absorption than a spray technique ("jetting"), although the maximum concentration of radiolabel in blood was reached more slowly. A biphasic curve characterized depletion over the first 5 days after treatment, and a half-life of 2 days was found. Continuous dermal absorption was observed after pour-on application. A small portion of the administered dose was recovered in urine and faeces.

The metabolic pathways in sheep were similar to those in rats. Metabolic conversion proceeds through opening of the cyclopropyl ring and oxidation of the α-carbon to a secondary propionic acid amide (N-[4,6-diamino-5-cyanopirimidin-2-yl]propionamide), or opening of the cyclopropyl ring and oxidation to a β-alanine derivative (3-[4,6-diamino-5-cyanopirimidin-2-y]amino]propionic acid) and dealkylation to descyclopropyl dicyclanil. The metabolism in sheep was determined by analysis of samples obtained from animals treated with a pour-on formulation containing [14C-pyrimidine]dicyclanil. Pooled extracts of urine, faeces, bile, excess material that had run off the animals during treatment, wool, fat, muscle, liver and kidney were analysed by one- and two-dimensional TLC and HPLC. In the two studies in which pour-on administration was used, 4–10% and 40–60% of the administered dose was recovered, respectively, in the run-off during the first hour after administration. In one study, about 4% of the dose retained on the skin was recovered in urine and faeces during the 7 days after dosing, while in the other study 0.6% of the retained dose was recovered within 48 h of administration. In urine, five fractions were identified, consisting of parent dicyclanil, N-(4,6-diamino-5-cyanopirimidin-2-yl)propionamide, descyclopropyl dicyclanil, 3-(4,6-diamino-5-cyanopirimidin-2-yl)amino]propionic acid, and an unidentified polar fraction from which descyclopropyl dicyclanil was released by microwave extraction. Dicyclanil and N-(4,6-diamino-5-cyanopirimidin-2-yl)propionamide accounted for 63% and 6% of the total residues in urine, respectively.

Only 1% of the total administered radiolabel was present in pooled faeces collected during 48 h after administration. Exhaustive extraction allowed recovery of 91% of the radiolabel in faeces, of which 72% was dicyclanil and 2% was descyclopropyl dicyclanil. In bile, most of the radiolabel appeared to be associated with strongly polar metabolites, although unchanged parent drug and descyclopropyl dicyclanil were also found.

Residue data
Sheep. In the studies in which [14C]dicyclanil was tested, high concentrations of radiolabel were extracted from muscle and fat at all times
after dosing, and less was extracted from liver and kidney. The main residue in muscle and fat tissues was the parent compound, which accounted for over 90% of the total residues. Dicyclanil and one unidentified metabolite were extracted from liver and kidney; kidney also contained descyclopropyl dicyclanil. Most of the extractable radiolabelled residues in liver and kidney were not characterized because of the extremely low levels of radioactivity. The radiolabel was widely distributed throughout the body, the concentration being highest in subcutaneous fat under the application area. Between day 7 and day 21 after treatment, the concentration of radiolabel decreased most sharply in blood and muscle and least in omental and subcutaneous fat.

The extractability of radiolabel from kidney and liver decreased as a function of time; for instance, 90% of the radiolabel in kidney was recovered on day 1 but only 50% on day 14. In the liver, 40–60% of the radiolabel was extractable, and an additional 20% could be extracted under harsh conditions. The extractable metabolites had a half-life of about 24 hours. The concentration of non-extractable residues in kidney, as dicyclanil equivalents, decreased from 0.009 to 0.006 mg/kg during the 3 days after administration. In liver, the concentration decreased from 0.07 to 0.02 mg/kg within 14 days after dosing. Overall, the parent drug accounted for about 15% of the total residues in liver and about 25% of those in kidney. In some samples, small amounts of descyclopropyl dicyclanil were found in kidney. Almost 100% of the radiolabel was extracted from adipose and muscle tissue and found to consist mainly of unchanged dicyclanil, with small amounts of descyclopropyl dicyclanil. A small amount of N-(4,6-diamino-5-cyanopyrimidin-2-yl)propionamide was found in muscle.

The effects of application technique, wool length, dose, breed, age and sex on residue depletion were studied with unlabelled dicyclanil in a total of 340 sheep and lambs in eight studies. Only the five studies that complied with GLP were considered relevant for the evaluation, as the others involved use of analytical methods with which only dicyclanil or both dicyclanil and descyclopropyl dicyclanil were determined. In the five studies used for the assessment, tissues were analysed for the presence of both dicyclanil and descyclopropyl dicyclanil. The tissue samples were collected at 2, 3, 7, 11, 14, 21, 28, 35, 42, 56, 58 and 84 days after administration in all the studies except one, in which they were collected at 7, 28, 56, 84 and 120 days after administration.

The recommended dose of the pour-on formulation, 0.1 g/kg of body weight, was used in only one study, while the other studies used 2–4
times the recommended dose. In the most comprehensive study, tissue samples were collected 7, 14, 21 and 35 days after dosing. The combined mean concentrations of dicyclanil and desclopropyl dicyclanil residues were expressed as dicyclanil equivalents and corrected for recovery and for the proportion of the total residues accounted for by the marker residue, determined in the studies with radiolabelled drug. The highest combined mean concentrations of dicyclanil and desclopropyl dicyclanil, expressed as dicyclanil equivalents, were 0.36 mg/kg in muscle, 6.3 mg/kg in liver, 3.3 mg/kg in kidney and 0.25 mg/kg in fat, measured 7 days after administration of the compound at the recommended dose. The respective values 35 days after dosing were 0.03, 0.38, 0.23 and 0.06 mg/kg.

The maximum concentrations of dicyclanil in the various tissues were generally found 3–14 days after administration, except in one study in which maximum concentrations of only 0.02–0.03 mg/kg were measured 56 days after administration. Concentrations of dicyclanil residues exceeding 0.1 mg/kg were found after administration at the recommended dose in only one study; however, the mean residue concentrations in most tissues were below 0.1 mg/kg 14 days after administration, although that in subcutaneous fat was 0.12 mg/kg 28 days after treatment.

**Analytical methods**

The concentrations of dicyclanil residues in muscle, liver, kidney and fat were determined by a fully validated HPLC method which allows separation of dicyclanil from its metabolite desclopropyl dicyclanil. Aqueous acetonitrile is used for the initial extraction and is followed by filtration. Separation of the lipids and successive purification steps are accomplished on various solid-phase extraction cartridges. Separation using HPLC is obtained by using a strong cation-exchange column with a mobile phase consisting of acetonitrile, sodium perchlorate and perchloric acid. Dicyclanil and desclopropyl dicyclanil elute at different times and are detected by their absorption of ultraviolet light at 270 nm. The limit of quantification for each compound is 0.01 mg/kg.

**Maximum Residue Limits**

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

- An ADI of 0–7 µg/kg of body weight, based on a toxicological endpoint, was established. This corresponds to a maximum ADI of 420 µg for a 60-kg person.
- The parent drug is the marker residue.
Table 3
Theoretical maximum daily intake of dicyclanil residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake (µg dicyclanil equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>200</td>
<td>200</td>
<td>60</td>
</tr>
<tr>
<td>Liver</td>
<td>400</td>
<td>2666&lt;sup&gt;b&lt;/sup&gt;</td>
<td>267</td>
</tr>
<tr>
<td>Kidney</td>
<td>400</td>
<td>1600&lt;sup&gt;d&lt;/sup&gt;</td>
<td>80</td>
</tr>
<tr>
<td>Fat</td>
<td>150</td>
<td>150</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>415</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as parent drug
<sup>b</sup> Based on a daily intake of 300g of muscle, 100g of liver, and 50g each of kidney and fat.
<sup>c</sup> The marker residue accounted for 15% of the total residues in liver.
<sup>d</sup> The marker residue accounted for 25% of the total residues in kidney.

- On the basis of the studies with radiolabelled cyhalothrin in sheep, the parent drug was estimated to account for 15% of the total residues in liver and 25% of those in kidney.
- An HPLC method for the detection of dicyclanil residues is available, which is suitable to meet regulatory needs, with a limit of quantification of 10µg/kg.

The Committee recommended MRLs in sheep of 200µg/kg for muscle, 400µg/kg for liver and kidney, and 150µg/kg for fat, expressed as parent drug.

From these values, the theoretical maximum daily intake of residues as dicyclanil equivalents from veterinary use is 415µg (Table 3). The Committee did not recommend an MRL for ewes’ milk as it did not consider the use of dicyclanil in lactating sheep.

3.3.5 Permethrin

Permethrin has not been evaluated previously by the Committee. At its present meeting, the Committee considered information on an 80:20 cis:trans isomeric mixture of permethrin which is used as an ectoparasiticide on cattle. The mixture is applied as a pour-on formulation at a dose rate of 4mg/kg of body weight up to a maximum of 1.6g per animal.

Information was provided on the depletion of residues of the 80:20 cis:trans isomeric mixture of permethrin after topical application at the recommended dose of 4mg/kg of body weight to calves and lactating cattle. Information was also provided on a proposed analytical method for regulatory purposes. The Committee was informed of an ongoing residue-depletion study in which the radiolabelled isomeric mixture was applied topically to cattle, which will provide information on the depletion of total residues and the parent drug. The biotrans-
formation of permethrin will also be examined in that study. The Committee noted that additional validation of the proposed analytical method is required.

The 40:60 cis:trans isomeric mixture of permethrin was evaluated by the 1982 Joint FAO/WHO Meeting on Pesticide Residues (11), which noted that different isomeric mixtures would require independent evaluation. The 1999 Joint FAO/WHO Meeting on Pesticide Residues (12) established an ADI of 0–0.05 mg/kg of body weight for technical-grade permethrin containing the cis- and trans-isomers in ratios of between 25:75 and 40:60.

At its present meeting, the Committee did not receive data to permit an evaluation of the toxicity of the 80:20 cis:trans isomeric mixture of permethrin intended for veterinary use. The Committee was aware that the cis-isomer is substantially more toxic than the trans-isomer when given as a single dose.

The Committee concluded that the available toxicological database was inadequate to allow assessment of the toxicity of the 80:20 cis:trans isomeric mixture proposed for use as a veterinary drug. In the absence of an ADI, the Committee was unable to recommend MRLs for this isomeric mixture of permethrin.

3.3.6 Metrifonate (trichlorfon)

Metrifonate (trichlorfon) is an organophosphonate pesticide with insecticidal, acaricidal and anthelmintic properties. It is used as an insecticide on food crops and forests. It is given orally, topically or parenterally for the control of endo- and ectoparasites in and on animals of various species. The recommended dose for treatment of cattle orally or topically with pour-on, wash or spray formulations is 50–75 mg/kg of body weight. Repeated dosing may be necessary. The preparations for use on horses are similar, but the recommended oral dose is 35 mg/kg of body weight. One topical formulation for use on horses also contains fentanil. The drug is also given orally to humans for infestation with Schistosoma haematobium, and has been studied for use in the treatment of Alzheimer disease.

Metrifonate has not previously been evaluated by the Committee. It was evaluated under the name “trichlorfon” on three occasions by the Joint FAO/WHO Meeting on Pesticide Residues (13–15), which established an ADI of 0–0.01 mg/kg of body weight in 1978 (15).

Toxicological data

The Committee considered the results of studies on the toxicokinetics, metabolism, acute, short-term and long-term toxicity,
genotoxicity, reproductive and developmental toxicity and neurotoxicity of metrifonate. It also considered studies of immune function and studies in humans. Most of the available studies were relatively old and many were published reports which lacked raw data or data on individual animals. Such studies are difficult to evaluate, and most could not be assessed independently. Unpublished reports of studies conducted during the 1980s and 1990s contained sufficient detail and were carried out according to appropriate standards for study protocol and conduct.

The absorption of metrifonate is rapid in all species tested, including humans, irrespective of the route of administration. Peak blood concentrations were achieved within 1–2 h but decreased quickly thereafter; the half-life of metrifonate in human plasma was approximately 2 h. The drug is widely distributed. Mefronate was detected in the milk of lactating cows, and the compound and its metabolites were found in fetal tissue in treated guinea-pigs. Mefronate undergoes conversion to dichlorvos via a dehydrochlorination reaction that occurs spontaneously at pH values above 5.5. Although little dichlorvos was recovered, it is generally believed to be responsible for the anticholinesterase effects of metrifonate. In mammals metrifonate is also metabolized via O-demethylation and cleavage of phosphorus–carbon bonds. The major metabolites are desmethyl metrifonate, desmethyl dichlorvos, dimethyl hydrogen phosphate, methyl dihydrogen phosphate and phosphoric acid. Excretion of metrifonate and its metabolites occurs primarily via the urine.

Mefronate is moderately toxic when given as a single oral dose, the LD₉₀ values being 620–950 mg/kg of body weight in mice, 140–660 mg/kg of body weight in rats, 160 mg/kg of body weight in rabbits, and 300–420 mg/kg of body weight in dogs. The signs of toxicity were indicative of inhibition of acetylcholinesterase activity, and the cause of death was usually respiratory failure. The rapid recovery observed after sublethal doses suggests that metrifonate is readily detoxified.

Mefronate was given orally to mice at doses of 10–750 mg/kg of body weight per day, to rats at doses of 0.05–250 mg/kg of body weight per day, and to dogs at doses of 0.5–45 mg/kg of body weight per day for periods of up to 16 weeks. The weights of the liver, kidney and spleen were increased at doses of 250 mg/kg of body weight per day and above in mice and at doses of 50 mg/kg of body weight per day and above in rats, but these changes were usually not associated with other evidence of toxicity, except in one study in rats in which hypertrophy of centrilobular hepatocytes and biochemical changes suggestive of altered lipid metabolism were seen. These effects occurred at
doses which also induced significant signs of cholinergic intoxication. Slight anaemia was observed in one study in rats at 100mg/kg of body weight per day and in one study in dogs at 45mg/kg of body weight per day. In one of three dogs given metrifonate at 45mg/kg of body weight per day, the prostate and testes were small and immature, and oligospermia was observed, but the animal had also lost weight and appeared to be severely stressed by the treatment. The NOELs for inhibition of brain and erythrocyte acetylcholinesterase activity were 15mg/kg of body weight per day in mice and 5mg/kg of body weight per day in rats; the NOEL for inhibition of erythrocyte acetylcholinesterase activity in dogs was 5mg/kg of body weight per day.

In two studies of up to 2 years' duration, mice were given metrifonate orally at 15–750mg/kg of body weight per day. Body-weight gain was impaired at doses of 30mg/kg of body weight per day and above. The mean weight of the liver was increased at a dose of 240mg/kg of body weight per day in one study, but no pathological alterations were found. The NOEL for inhibition of brain and erythrocyte acetylcholinesterase activity was 49mg/kg of body weight per day. The incidences of tumours were unaffected by treatment. Neither study was suitable for identifying a NOEL for establishing an ADI.

Six studies of up to 2 years' duration were conducted in which rats were given metrifonate in the diet at 2.5–160mg/kg of body weight per day. The weights of the liver and spleen were increased in three studies at 50mg/kg of body weight per day and above. Hypercholesterolaemia, duodenal hyperplasia, and gastritis of the non-glandular stomach were seen at 13mg/kg of body weight per day and above. Anaemia, enhancement of age-related nephropathy, increased hyperplasia of the renal tubules, degeneration of the stomach tunica muscularis, and inflammation and hyperplasia of the lungs were observed at 76mg/kg of body weight per day and above. Other observations included ovarian atrophy at 20mg/kg of body weight per day and above, depression of spermatogenesis at 50mg/kg of body weight per day, and a slight increase in the incidence of benign mammary tumours in females with a reduction in the time to appearance at 20mg/kg of body weight per day and above. The NOEL for inhibition of brain and erythrocyte acetylcholinesterase activity was 13mg/kg of body weight per day. The incidence of mammary tumours was slightly increased in female Sprague–Dawley rats in two studies. However, the Committee concluded that metrifonate does not have carcinogenic potential in rats, as the tumours were benign, this strain of rats has a relatively high and variable background incidence of mammary tumours, and the finding was not replicated in several other studies in various species. The overall NOEL in rats was 4.5mg/kg of
body weight per day, on the basis of the hypercholesterolaemia and pathological changes to the gastrointestinal tract.

In two studies of 1 and 4 years’ duration, dogs were given metrifonate orally at doses of 1.3–80 mg/kg of body weight per day. Deaths occurred at doses of 20 mg/kg of body weight per day and above, and signs of hepatocellular damage and increased severity of inflammation were seen in the liver at doses of 25 mg/kg of body weight per day and above. In animals given 20–25 mg/kg of body weight per day, the weight of the spleen was increased, with atrophy of lymphoid tissue; small ovaries, reduced testis weight, and decreased spermatogenesis were also observed. “Blood cholinesterase” activity was inhibited at doses of 5 mg/kg of body weight per day and above. The overall NOEL in dogs was 1.3 mg/kg of body weight per day, on the basis of inhibition of cholinesterase activity.

Metrifonate was administered orally to rhesus monkeys at a dose of 0, 0.2, 1 or 5 mg/kg of body weight per day for 10 years. Inhibition of plasma, erythrocyte and brain cholinesterase activities was seen at 1 and 5 mg/kg of body weight per day; animals in the highest-dose group also showed clinical signs of toxicity indicative of cholinesterase inhibition, reduced body-weight gain and anaemia. In a separate 6-month study in rhesus monkeys, erythrocyte acetylcholinesterase activity was not depressed at 0.2 mg/kg of body weight per day, the highest dose tested. The NOEL in monkeys was 0.2 mg/kg of body weight per day, on the basis of inhibition of brain and erythrocyte acetylcholinesterase activity.

Metrifonate has been tested in a wide range of assays for genotoxicity and chromosomal-damaging activity, with considerable variation in the results. Metrifonate induced point mutations in microorganisms and cultured mammalian cells, DNA damage in microorganisms, and chromosomal aberrations and sister chromosome exchanges in cultured mammalian cells. The results of tests for point mutation in Drosophila melanogaster and for sister chromatid exchange in bone marrow were negative. Although positive results were obtained in a few assays for chromosomal damage in bone marrow and the germ cells of male animals exposed to near-lethal doses, the results of most studies of micronucleus formation, metaphase alterations, and dominant lethal mutations were negative. Since the tests conducted in vivo produced mostly negative results when metrifonate was administered orally, the Committee considered that the weight of evidence indicates that metrifonate is unlikely to have genotoxic potential.

Metrifonate was given in the diet to rats at doses equivalent to 0, 10, 30, 100 or 300 mg/kg of body weight per day in a three-generation
study of reproductive toxicity, with two litters per generation. The body-weight gain of F₀ dams at 100 and 300mg/kg of body weight per day was depressed. The slight effects on the gonads reported in the studies of general toxicity were not consistently reflected in this study. The pregnancy rate of rats of the F₁ generation at 100 and 300mg/kg of body weight per day was reduced, but the fertility of other generations was unaffected. The litter size of dams in the highest-dose group was reduced and all of the offspring died, but reproductive parameters were unaffected at lower doses. The NOEL was 30mg/kg of body weight per day in adults, on the basis of reduced body-weight gain, and 100mg/kg of body weight per day for reproductive effects, on the basis of reduced litter size and the death of offspring.

Two studies of developmental toxicity were conducted in mice given metrifonate by gavage at 200–600mg/kg of body weight per day. Maternal toxicity in the form of reduced body-weight gain was observed in all treated groups; animals dosed at 300mg/kg of body weight per day and above also showed an increased mortality rate. The effects of these maternally toxic doses included increased embryotoxicity, decreased fetal weight, and delayed fetal development. The incidence of cleft palate was slightly increased at 500 and 600mg/kg of body weight per day. NOELs were not identified for maternal toxicity, embryotoxicity or fetotoxicity.

Studies of developmental toxicity were conducted in rats given metrifonate in the diet or by gavage at doses of 8–520mg/kg of body weight per day. In three studies, maternal toxicity in the form of reduced body-weight gain and/or an increased mortality rate was observed at doses of 150mg/kg of body weight per day and above. The NOEL for maternal toxicity was 75mg/kg of body weight per day. Fetotoxic effects included reduced fetal body weight at 75mg/kg of body weight per day, minor skeletal changes at 380mg/kg of body weight per day, and an increased incidence of fetal malformations such as morphological alterations of the skull, central nervous system and limbs, micrognathia, cleft palate, facial haematomas, generalized oedema, and defects in major blood vessels at doses of 430mg/kg of body weight per day and above. In three other studies, the incidence of fetal malformations was unaffected. The NOEL for developmental effects was 50mg/kg of body weight per day, on the basis of fetotoxicity.

In a study in hamsters, metrifonate was given by gavage at a dose of 0, 200, 300 or 400mg/kg of body weight per day during pregnancy. Signs of cholinesterase inhibition were observed in all treated groups; those dosed at 300 and 400mg/kg of body weight per day also had
depressed body-weight gain. The NOEL for maternal toxicity was 100 mg/kg of body weight per day. Fetal body weight was decreased at 300 and 400 mg/kg of body weight per day, and an increased incidence of fetal stunting was seen at the highest dose. The NOEL for developmental effects was 200 mg/kg of body weight per day.

Guinea-pigs given metrifonate by gavage at a dose of 100 mg/kg of body weight per day for 6 days during gestation had abortions and stillborn fetuses, and the offspring had reduced body weight and brain weight, locomotor disturbances, and morphological and biochemical alterations in the brain.

In two studies of developmental toxicity in rabbits, metrifonate was given by gavage at doses of 5–110 mg/kg of body weight per day. Overt toxicity and inhibition of erythrocyte acetylcholinesterase activity were observed in dams given doses of 35 mg/kg of body weight per day and above, and body-weight gain was affected at all doses. The rate of fetal resorption was slightly increased and fetal development retarded at the highest dose, but no fetal abnormalities were found. A NOEL for maternal toxicity was not identified. The NOEL for developmental effects was 45 mg/kg of body weight per day.

Several outbreaks of congenital tremor with cerebellar hypoplasia were reported in piglets of sows that had been treated with metrifonate, and the syndrome was subsequently reproduced experimentally. The piglets of sows treated with metrifonate in the diet at 50–100 mg/kg of body weight per day 55–98 days after conception showed neurological signs and hypoplasia and loss of Purkinje cells in the cerebellum.

Rats given metrifonate in the diet at a dose equivalent to 200 mg/kg of body weight per day for 13 weeks showed decreased locomotor activity and loss of coordination. In another feeding study in rats, a dose equivalent to 30 mg/kg of body weight per day for 3 weeks increased locomotor activity and impaired learning ability and nerve conduction.

The results of numerous studies in hens consistently demonstrate the acute neurotoxicity of metrifonate. Delayed neurotoxicity, associated with degeneration of nervous tissue and marked inhibition of neuropathy target esterase, was not seen in hens. However, a monkey (species unspecified) given a single oral dose of 250 mg/kg of body weight showed impaired nerve conduction 4 weeks after treatment and histological evidence of demyelination and axonal degeneration.

Metrifonate has been used as an anthelmintic agent in humans. Oral doses of up to 10 mg/kg of body weight given on 2–4 occasions were
well tolerated, with few clinical symptoms of toxicity. Oral administration of a dose of 24mg/kg of body weight caused cholinergic symptoms, but the effects were not cumulative. In a few cases, spermatogenesis appeared to have been impaired. The results of clinical trials with metrifonate in the treatment of Alzheimer disease showed dose-related but reversible muscular weakness. The recommended dose of 0.5-0.9mg/kg of body weight resulted in a small increase in the frequency of generalized weakness and fatigue, while doses of 1.25mg/kg of body weight and higher produced significant weakness. Dose-related inhibition of erythrocyte acetylcholinesterase activity was also observed in these studies.

In 121 subjects, an initial oral dose of 0.5mg/kg of body weight per day for 2 weeks inhibited erythrocyte acetylcholinesterase activity by 29%. Subsequent administration of an oral dose of 0.2mg/kg of body weight per day for 10 weeks maintained the level of inhibition of erythrocyte acetylcholinesterase activity at 30-37%, with no increase with increased duration of dosing. Since this dose enhanced the inhibition caused by the initial dose by only 8%, a non-significant change, the Committee concluded that the NOEL was 0.2mg/kg of body weight per day.

In some cases of severe human poisoning with metrifonate, weakness and loss of feeling in the extremities, difficulty in walking, muscular atrophy, and motor nerve damage have been observed. In many of these cases, the doses might have been lethal in the absence of medical intervention. The Committee concluded that extremely high doses of metrifonate would be required to achieve the level of inhibition of neuropathy target esterase associated with delayed neurotoxicity.

The Committee concluded that inhibition of acetylcholinesterase activity was the most relevant end-point for establishing an ADI for metrifonate. The most appropriate NOEL was 0.2mg/kg of body weight per day for inhibition of erythrocyte acetylcholinesterase activity in humans treated orally. A safety factor of 10 was applied to this figure, giving an ADI of 0-20µg/kg of body weight.

**Pharmacokinetic data**

**Cattle.** Metylflonate labelled with 14C on the α-carbon of the trichloroethyl group was applied to the backs of eight calves at a target dose of 40mg/kg of body weight, and one male and one female were slaughtered on each of days 1, 2, 3 and 5 after treatment. Between 14 and 49% of the dose was not available for absorption because of run-off from the backs of the treated animals. When the animals were washed after slaughter, the radiolabel in the washings
represented 4–16% of the administered dose. The amount remaining on the skin represented 8–28% of the dose and would have continued to contribute to the tissue residues while it was absorbed. Only 2 and 6% of the initial dose was estimated to have been absorbed by the two calves slaughtered 5 days after dosing.

The pharmacokinetics of metrifonate in plasma, urine and faeces was measured in the two calves slaughtered 5 days after treatment. Absorption was rapid; the maximum concentration of total radiolabel in plasma (C_{max}), expressed as metrifonate equivalents, was 1.0 mg/kg at 4 h after treatment in the male and 0.36 mg/kg at 6 h in the female. Thereafter, the C_{max} declined in a biphasic manner, rapidly up to 24 h and more slowly between 24 and 120 h after dosing. The half-lives for elimination of total radiolabel into the plasma of the two calves were 124 and 258 h, respectively. Relatively little of the radiolabel was excreted: 2.8 and 1.6% of the administered dose was excreted in urine and 3.3 and 0.3% in the faeces of the two calves, respectively. If account is taken of the significant portion of the administered dose that was not available for absorption, the amount excreted is higher, expressed as a percentage of the retained dose. Over the 5 days after treatment, 14 and 5.2% of the retained dose was excreted in urine and 16 and 1.0% in the faeces of the two calves, respectively.

The metabolic profiles of topically administered metrifonate were investigated in tissues from the two calves slaughtered on day 1 after dosing. Extensive extraction procedures resulted in extraction of 83–97% of the total residues in muscle, liver and kidney and 73–85% of those in fat. Identification of metabolites in the extractable fractions proved to be difficult, because of the complex nature of the residues. In all samples of fat taken on day 1 after dosing, metrifonate and dichloroacetic acid were identified as the major metabolites. Dichlorvos was found in one sample. Liver and kidney contained mainly polar and other unknown metabolites. Muscle contained metrifonate, but the amounts were not quantified. Since neither metrifonate nor its major metabolites were quantified in any tissue, the proportion of the total residues represented by a single compound such as metrifonate could not be calculated. A similar pattern of biotransformation was found in tissues from the other six calves.

In a study conducted in 1952, a lactating dairy cow infested with “grubs” (not specified) was given [^{32}P]metrifonate orally at a dose of 25 mg/kg of body weight. Samples of blood, urine, faeces and milk were collected, and the concentrations of total radiolabel and that associated with metrifonate and dichlorvos were measured. The C_{max} in blood, 15 mg/kg, expressed as metrifonate equivalents, was attained
2h after dosing; 7.5% was accounted for by metrifonate. Most (66%) of the radiolabel was eliminated in urine within 12h, but only 0.26% of the excreted radiolabelled residues was associated with metrifonate. A major metabolite, which accounted for 73% of the excreted radiolabelled residues, was tentatively identified as a dehydrochlorination product of the two possible glucuronides of metrifonate. Less than 3% of the radiolabel was excreted in faeces. Dichlorvos was not detected in any of the samples.

*Goats.* The metabolic fate of [${}^{14}$C]metrifonate given orally at a dose of 8.6 mg/kg of body weight per day on 3 consecutive days was studied in two lactating goats. The goats were killed 4h after the last dose, and samples of tissues and milk were collected and analysed for metabolites. Unmetabolized metrifonate accounted for 6–7% of the total residues in muscle and kidney, but was not present in liver, fat or milk. A large proportion of the radiolabel was incorporated into tissue proteins and sugars and accounted for 38% of the total residues in muscle, 52% in liver, and 23% in kidney. The conjugates of dichloroacetic acid accounted for 43% of the total residues in muscle, 11% in liver, 44% in kidney, and 70% in fat. Other metabolites, including desmethylmetrifonate, desmethyldichlorvos and glucuronyl metrifonate, were identified at low levels.

*Residue data*

*Cattle.* In the study described above in which [${}^{14}$C]metrifonate was applied topically to the backs of eight calves at a target dose of 40 mg/kg of body weight, samples of fat, muscle, and skin close to and distant from the application site were collected at slaughter. The concentration of radiolabel in plasma and in tissues distant from the treated area appeared to reach a maximum at 3 days and declined thereafter. The concentrations of total residues in the tissues of animals slaughtered 1, 2, 3 and 5 days after dosing, expressed as the mean metrifonate equivalents in two samples of each tissue, were respectively 100, 220, 180 and 92 µg/kg in muscle; 590, 1300, 2000 and 940 µg/kg in liver; and 470, 730, 1200 and 560 µg/kg in kidney. The concentrations of total residues in various fats 1 day after treatment, expressed as metrifonate equivalents, were 22 µg/kg in renal fat, 42 µg/kg in omental fat, and 970 and 110 µg/kg in subcutaneous fat close to and distant from the application site, respectively. At days 2, 3 and 5, the concentrations of total residues in fat (combined) were 810, 950 and 200 µg/kg, respectively.

Two new studies that complied with GLP were evaluated in which the residues of metrifonate and dichlorvos were measured in the tissues and milk of cattle after topical application of unlabelled metrifonate.
Adult cattle were treated with a single spray of a 5% solution of metrifonate at a dose of 40mg/kg of body weight, and killed in groups (two males and two females) 12h and 1, 3 and 7 days after treatment. Samples of liver and kidney, and of muscle and fat close to and distant from the area of application were collected and analysed for residues of both metrifonate and dichlorvos by a validated liquid chromatography–tandem MS method. No residues of metrifonate were detected (limit of quantification, 50µg/kg) at any time in muscle or fat distant from the area of application or in liver or kidney. However, 12h after dosing, residues of metrifonate were found at concentrations of 51–170µg/kg in subcutaneous fat close to the treated area in samples from all four animals. Residues of dichlorvos were found at a concentration of 140µg/kg only in fat tissue close to the treated site in a sample taken from one animal on day 1, and residues of metrifonate were found in samples of muscle (at 90µg/kg) and fat (at 2350µg/kg) from this animal.

In the second study, eight dairy cows were treated once with a spray of a 5% solution of metrifonate at a dose of 40mg/kg of body weight, and the concentration of metrifonate in milk was measured by a validated liquid chromatography–tandem MS method. Most of the metrifonate was excreted in milk during the first 12h after treatment. The mean concentrations (± standard deviation) were 79 ± 57µg/kg 6h after dosing and 61 ± 84µg/kg 12h after dosing; the highest concentration was 200µg/kg in the milk from one cow 6h after dosing. The concentrations of residues were just above the limit of quantification (25µg/kg) in one cow (28µg/kg) at 24h and in another cow (29µg/kg) at 36h, but thereafter no residues were detected (limit of detection, 2.5µg/kg).

Three horses were given single oral doses of a paste containing both metrifonate and febantel at 35 and 6mg/kg of body weight, respectively. The horses were killed 14 days later, and samples of fat and muscle were assayed for the sum of metrifonate and dichlorvos measured as the common breakdown product, dimethyl phosphite, by gas chromatography. No residues were detected at concentrations above the limit of quantification of the analytical method (50µg/kg).

**Analytical methods**

A validated GC–MS method has been reported for the identification and quantification of metrifonate residues in liver, kidney, muscle and fat of cattle, using d₆-metrifonate as an internal standard. A modification of this method has been validated for the analysis of metrifonate residues in the edible tissues of cattle and horses and in cows’ milk.
In the modified method, residues of metrifonate are extracted from tissues and milk using acetonitrile containing 0.1% formic acid. Samples are purified by applying the extracts to a silica cartridge, washing the cartridge with ethyl acetate:heptane (1:9), and eluting the metrifonate fraction with ethyl acetate. GC–MS is performed with either an internal standard (d₅-metrifonate) or an external standard. Selective ion monitoring is then used to detect metrifonate and d₅-metrifonate at m/z 109 and 115 atomic mass units, respectively.

The method has been validated for use over the linear range of the detector response, which is 0.01–1 mg/kg for tissues and 0.05–2 mg/kg for milk. Using the detector in the selective ion-monitoring mode with an established gas chromatography retention time makes it highly likely that the method is specific. The recovery of metrifonate in all tissues is 70–112%. The limit of detection of the method in cattle is 9 μg/kg for liver and muscle, 6 μg/kg for kidney and fat, and 2 μg/kg for milk. In horses, the limit of detection is 8 μg/kg for muscle, 20 μg/kg for liver, 16 μg/kg for kidney, and 9 μg/kg for fat. The limit of quantification is 50 μg/kg for all tissues and 25 μg/kg for milk.

No interference from the matrix has been reported in this method, which was proposed by the sponsor for use in routine surveillance. As the internal standard is not available commercially, either external standardization should be used or the introduction of a suitable surrogate standard should be investigated.

**Maximum Residue Limits**

In reaching its decision on MRLs for metrifonate, the Committee took the following factors into account:

- The ADI is 0–20 μg/kg of body weight, which is equivalent to a maximum ADI of 1200 μg for a 60-kg person.
- Only metrifonate and dichlorvos are of toxicological concern. Mefenofos is a pro-drug, and dichlorvos is the only metabolite with effective insecticidal action.
- Dichlorvos is highly unstable and is not present in the edible tissues or milk of food-producing animals.
- The metabolism of metrifonate is broadly similar in target and laboratory animals.
- The parent drug is the marker residue.
- Mefenofos is metabolized so extensively that the proportion of the total residues represented by the residue marker cannot be determined.
• There is a suitable analytical method available for routine determination of metrifonate, with a limit of quantification of 50 μg/kg for muscle, liver, kidney and fat and 25 μg/kg for milk.

• The concentrations of metrifonate in muscle and fat samples distant from the site of application were below the limit of quantification at all times. Within 1 day of administration of a pour-on preparation, the concentrations of residues in muscle and fat samples collected close to the site of application were above the limit of quantification in only a few animals. However, no residues were present in fat or muscle close to the site of administration by 3 days after treatment.

• Residues of metrifonate were found at concentrations above the limit of quantification in milk (25 μg/kg) from the first three milkings after treatment, but thereafter the concentrations were below the limit of quantification. No residues of dichlorvos were found at concentrations greater than the limit of quantification (25 μg/kg) in any sample of milk.

Residues of metrifonate were not detected in the residue-depletion studies reviewed by the Committee. The Committee concluded that residues of metrifonate would not be found in muscle, liver, kidney or fat at the limit of quantification of the available analytical methods. Therefore, the Committee recommended that MRLs should not be allocated for muscle, liver, kidney and fat, since no detectable residues should be present in tissues from animals treated with metrifonate in accordance with good practice in the use of veterinary drugs. The limit of quantification may be used by national governments as a guide for the maximum concentrations in muscle, liver, kidney and fat of cattle. The guidance values are 50 μg/kg for muscle, liver, kidney and fat in cattle, expressed as parent drug. Insufficient information was available to extend these MRLs to horses.

The Committee recommended an MRL of 50 μg/kg for cows' milk, expressed as parent drug.

From this MRL, the theoretical maximum daily intake of metrifonate residues would be 75 μg, based on a daily consumption of 1.5 kg of milk. This is equivalent to only 6% of the maximum ADI.

3.4 Production aid

3.4.1 Melengestrol acetate

Melengestrol acetate is a synthetic progestogen which is active after oral administration. It is used to improve the efficiency of feed conversion, promote growth, and suppress estrus in female beef cattle. The range of approved doses is 0.25–0.50 mg/heifer per day. The drug can be administered alone or in combination with other growth-promoting
drugs. Melengestrol acetate is fed for the duration of the fattening and finishing period, usually for 90–150 days.

Melengestrol acetate has not previously been evaluated by the Committee.

Toxicological data
The Committee considered information from a range of studies on melengestrol acetate, including studies on its pharmacokinetics, biotransformation, acute, short-term and long-term toxicity, carcinogenicity, genotoxicity, and reproductive and developmental toxicity. It also considered the results of studies in humans. Most of the studies were conducted before 1979 according to the standards in existence at that time and were not carried out in compliance with GLP. More recent studies were conducted according to the appropriate standards for study protocol and conduct.

The results of limited studies of the pharmacokinetics of melengestrol acetate in rabbits and humans have been reported. The bioavailability of melengestrol acetate after oral administration and its kinetics in plasma have not been determined. In studies in which radiolabelled melengestrol acetate was used, $^3$H or $^{14}$C was inserted at the 6-methyl position. In rabbits, 59% of an orally administered dose of $^{14}$Cmelengestrol was excreted within 7 days in urine and faeces at a ratio of about 1:3, with a peak elimination rate on the first day. In women, the excretion of $^{14}$Cmelengestrol acetate was complete within 10 days, and 74% of the radiolabel was recovered in urine and faeces. The half-life estimated from the data on excretion was 3–5 days.

Limited information was available on the biotransformation of melengestrol acetate in cattle, rabbits and humans in vivo and in cattle and rat liver microsomes in vitro. Melengestrol acetate is extensively metabolized, with the formation of numerous metabolites. The metabolites have been neither adequately identified nor characterized with respect to their biological activity. In cattle, intact melengestrol acetate accounted for up to 86% and 29% of the total radiolabel in fat and liver, respectively. In cattle and rat liver microsomes, several mono- and dihydroxylated metabolites were identified. In the urine of rabbits, two-thirds of the radiolabel was found as glucuronides. The 6-methyl-hydroxylated metabolite was identified in the free and conjugated forms as one of the major metabolites. In humans, 68% of the radiolabel in urine was associated with conjugates, whereas faeces contained more unconjugated compounds. Peaks representing 13 metabolites with an intact steroid nucleus were detected, one of which was identified as 2α-monohydroxylated melengestrol acetate.
Melengestrol acetate has little toxicity after a single dose, although the studies of acute toxicity were limited, since a large volume of the vehicle had to be administered. The LD₅₀ values after intraperitoneal injection were >2.5 g/kg of body weight in mice and >2 g/kg of body weight in rats. No deaths were observed among rats given doses of 8 g/kg of body weight orally or 5 g/kg of body weight subcutaneously. Dermal application to the intact or abraded skin of rabbits at the maximum achievable dose of 22 mg/kg of body weight caused no toxic reaction.

Short-term tests of the toxicity of melengestrol acetate have been performed in mice, rats, rabbits, dogs and monkeys. Melengestrol acetate had a greater effect in females than in males, with hormonal (progestational and corticosteroidal) effects as the most sensitive end-points.

In TUC/ICR mice of each sex that received melengestrol acetate orally at a dose of 0, 1, 3, 10 or 30 mg/kg of body weight per day for 30 days, the body weights were slightly increased at 3 mg/kg of body weight per day but were decreased at higher doses. Changes in female reproductive organs, such as decreased weights of the ovaries and uterus at the highest dose and the absence of corpora lutea at doses of 3 mg/kg of body weight per day and above were considered to be progestational changes. The NOEL for hormonal effects was 1 mg/kg of body weight per day.

In a 21-day study, puberal female C₅77Han/f mice received melengestrol acetate in the diet at concentrations equal to 0, 0.05, 0.25, 0.5, 1.5, 2.5, 5 or 25 mg/kg of body weight per day. Body weight was significantly increased at doses of 2.5 mg/kg of body weight per day and above, and the serum concentration of prolactin and the weight of the uterus but not the ovaries were increased at the highest dose. The NOEL was 1.5 mg/kg of body weight per day.

In mature female ICR and C₅77Han/f mice given an oral dose of 0, 0.25, 0.5, 2.5, 5, 10, 15, 20, 25 or 40 mg/kg of body weight per day for 20 days, melengestrol acetate caused a significant, dose-related increase in mammary duct proliferation in C₅77Han/f mice at doses of 15 mg/kg of body weight per day and above. The drug had no effect on mammary duct proliferation in ICR mice.

In order to elucidate the contribution of increased serum prolactin concentration to melengestrol acetate-induced mammary duct proliferation, groups of weanling female C₅77Han/f mice were given diets containing melengestrol acetate at concentrations providing doses of 0, 0.5, 1.5, 2.5, 5, 10 or 25 mg/kg of body weight per day for 20
days with or without the prolactin inhibitor 6-methyl-8β-ergoline-acetonitrile. The serum prolactin concentration and mammary duct proliferation were enhanced at all doses, and the effects were partially inhibited by 6-methyl-8β-ergoline-acetonitrile. There was no statistically significant association between mammary duct proliferation and serum prolactin concentration. A NOEL could not be identified.

In juvenile rats given melengestrol acetate for 28 days by gavage at a dose of 0, 1, 3 or 10 mg/kg of body weight per day, food consumption and body weight were reduced in all treated animals. Haematological changes were also seen, which included a dose-related increase in the erythrocyte volume fraction and a decreased leukocyte count in animals at the highest dose. In females, the weights of the adrenals, uterus and ovaries were reduced at all doses, and this effect was associated with atrophy of these organs and the absence of corpora lutea in most animals. In males, atrophy of the adrenal and accessory sex glands was observed only at 3 and 10 mg/kg of body weight per day. The effects reported are consistent with progestational and corticosteroidal activity. A NOEL could not be identified.

In a 90-day study of toxicity, rats received melengestrol acetate in their diet at concentrations providing 0, 0.015, 0.15 or 0.3 mg/kg of body weight per day. The serum cholesterol concentration was increased in females at 0.15 and 0.3 mg/kg of body weight per day. Changes characteristic of the hormonal effects of melengestrol acetate were observed, such as: decreased weights of the adrenals, ovaries and uterus at 0.3 mg/kg of body weight per day; hyperplasia of the mammary glands and endometrium, agenesis of the corpora lutea, and bone-marrow hypoplasia at 0.15 and 0.3 mg/kg of body weight per day; and enlargement of the mammary glands at 0.015 mg/kg of body weight per day. Other effects observed at the lowest dose, although not statistically significant, were consistent with the changes seen at higher doses. The Committee concluded that 0.015 mg/kg of body weight per day was the minimally effective dose.

In another 90-day study, weanling rats were fed diets containing melengestrol acetate at 0 or 0.055 mg/kg of body weight per day. The treatment-related effects observed were slight increases in erythrocyte volume fraction, erythrocyte count and haemoglobin concentration, and significantly lower adrenal, ovarian and testicular weights. A NOEL could not be identified.

Rabbits were injected intramuscularly with melengestrol acetate at 20 mg/kg of body weight every second day for 22 days. All animals lost weight and had diarrhoea. Haematological evaluation revealed
decreased leukocyte counts and impaired platelet function. All four males died during the last week of treatment from thoracic bleeding after blood sampling. At termination of the study, serum cholesterol concentrations and the activities of aspartate aminotransferase, lactate dehydrogenase and alkaline phosphatase were increased in the four surviving females. At necropsy, these animals were found to have muscular atrophy, small adrenals, and enlarged livers with swollen hepatocytes containing glycogen deposits.

Groups of two male and two female beagle dogs were given melengestrol acetate in gelatin capsules orally at a dose of 0, 1, 3 or 10mg/kg of body weight per day for 29 days. Treatment at 3 and 10mg/kg of body weight per day induced slight-to-moderate diuresis, with urine of decreased specific gravity. Body weight was slightly decreased and food consumption increased in all treated animals. Small increases were observed in the activity of serum alkaline phosphatase at 3 and 10mg/kg of body weight per day and of serum alanine aminotransferase at 10mg/kg of body weight per day. A dose-related decrease in adrenal weight and increase in liver weight were seen, with histopathological changes indicative of glycogen deposition. A NOEL was not identified.

Groups of eight adult female rhesus monkeys were treated orally with melengestrol acetate at a dose of 0, 1.5, 15, 75 or 150µg/kg of body weight per day for one menstrual cycle. Ovulation was monitored by measuring the periovulatory surge of luteinizing hormone and the decrease in estrogen concentration and confirmed by laparoscopy. The number of monkeys that ovulated decreased significantly during treatment, from 88% in controls and the lowest-dose group, to 38, 25 and 12% at 15, 75 and 150µg/kg of body weight per day, respectively. The menstrual cycle was prolonged at 75 and 150µg/kg of body weight per day, but melengestrol acetate had no significant effect on the serum concentrations of progesterone and estrogens (estradiol-17β and estrone). Changes in the periovulatory surge of luteinizing hormone and the suppression of ovulation were the most sensitive endpoints in this study. The NOEL for suppression of ovulation was 1.5µg/kg of body weight per day.

In a range-finding study for hormonal effects, groups of six female cynomolgus monkeys were treated orally with melengestrol acetate by nasogastric intubation at a dose of 0, 2.5, 5 or 10µg/kg of body weight per day for one menstrual cycle. One monkey in the lowest-dose group and one in the highest-dose group were withdrawn from the study because they showed anorexia. One monkey from each of the groups dosed at 5 and 10µg/kg of body weight per day failed to
ovulate during treatment. Two monkeys from each of the groups dosed at 2.5 and 10μg/kg of body weight per day had prolonged menstrual cycles; one animal from the group dosed at 5μg/kg of body weight per day and one control were also affected. No consistent dose–response relationship was seen for effects on hormone concentrations. The serum concentration of estradiol was decreased during the luteal phase of the menstrual cycle in animals at 5 and 10μg/kg of body weight per day, and that of luteinizing hormone was suppressed at 2.5 and 5μg/kg of body weight per day. Melengestrol acetate had no consistent effect on the serum concentrations of progesterone and follicle-stimulating hormone. The authors concluded that “melengestrol acetate may have exerted subtle effects on the menstrual cycle of cynomolgus monkeys”.

In a follow-up study, female cynomolgus monkeys were given melengestrol acetate at a dose of 0, 5, 10 or 25μg/kg of body weight per day for three consecutive menstrual cycles, up to a maximum of 105 days. Groups of eight animals were observed for three consecutive menstrual cycles before treatment. Two animals (one from each of the groups dosed at 5 and 10μg/kg of body weight per day) were not included in the final evaluation because they had abnormal cycles before treatment. The occurrence of ovulation was determined by observing the periovulatory surge of luteinizing hormone, the peak of estradiol, and the increase in progesterone concentration in the luteal phase. The hormonal and menstrual cycle variables showed the changes that would be expected to be induced by a progestogen, such as significantly decreased serum concentrations of luteinizing hormone and estradiol at 10 and 25μg/kg of body weight per day and of progesterone at 25μg/kg of body weight per day. Significantly fewer animals in the highest-dose group menstruated and ovulated, and significantly more animals in the groups dosed at 10 and 25μg/kg of body weight per day had changed cycles. In the remaining animals, the dose-related prolongation of the first menstrual cycle did not reach statistical significance. The serum concentrations of follicle-stimulating hormone and cortisol were not affected by melengestrol acetate. The effects at 5μg/kg of body weight per day, although not statistically significant, were consistent with the hormonal response seen at higher doses. The Committee considered that 5μg/kg of body weight per day was the minimally effective dose and was close to the NOEL for hormonal effects.

In heifers fed melengestrol acetate at a dose equal to 0.16μg/kg of body weight per day for 15–116 days after estrus, treatment reduced the number of animals in estrus by 40%, and doses equal to 0.7 and 1.1μg/kg of body weight per day consistently suppressed estrus in all
animals. Melengestrol acetate was also fed to heifers at a dose equal to 1.8 μg/kg of body weight per day from 2.5 to 11.3 months of age. When the animals reached maturity, the serum concentrations of estradiol-17β and estrone were significantly increased over those in controls, and that of progesterone was suppressed to values similar to those occurring in the prooestrus period. The serum concentrations of cortisol and corticosterone were depressed to about 50% of those in untreated animals. A NOEL could not be identified for the progestational and corticosteroidal activity of melengestrol in cattle.

In a study of carcinogenicity, ICR mice received diets containing melengestrol acetate at concentrations providing doses equal to 0, 0.017 or 17 mg/kg of body weight per day for up to 24.5 months. The animals in the highest-dose group weighed more than controls throughout the study, and their survival rate was significantly lower. These effects were attributed to the stress of obesity caused by melengestrol acetate. The incidence of benign and malignant tumours was reduced in treated females but not males. A slight, non-significant increase in the incidence of mammary adenocarcinomas was observed in animals in the highest-dose group. No firm conclusion could be drawn about the carcinogenic potential of melengestrol acetate in ICR mice.

In a similar study, prepuberal C3H/An/f mice, which were previously shown to be more sensitive than ICR mice to the effects of melengestrol acetate on mammary duct proliferation, were given diets containing melengestrol acetate at concentrations providing doses equal to 0, 0.017 or 17 mg/kg of body weight per day for up to 35 months. Females in the highest-dose group had an increased incidence of malignant tumours, consisting mainly of mammary adenocarcinomas. This increase was assumed to be due not to a direct carcinogenic effect of melengestrol acetate but to the promoting effect of increased prolactin concentrations.

In another study, five groups of mature C3H/An/f mice aged 63–84, 77–91, 84–105, 98–112 and 119–126 days were used to assess the effect of age on the development of melengestrol acetate-induced mammary tumours. The animals received a diet containing melengestrol acetate at concentrations providing doses equivalent to 0, 0.5, 1, 1.5, 2.5, 5, 10, 15 or 25 mg/kg of body weight per day. The study was terminated after 27 months, when the mortality rate reached 90%. Age had a significant effect on the development of mammary tumours in both treated and control mice, with the greatest incidence in the youngest group. Except for a lower incidence of mammary tumours in the group that received 10 mg/kg of body weight per day, the incidence
increased in a dose-related manner from 1.5 mg/kg of body weight per day. Melengestrol acetate had no effect on the time at which tumours were first detected. The treatment-related non-neoplastic lesions that were observed consisted of progestational effects, such as increased cystic endometrial hyperplasia at doses of 5 mg/kg of body weight per day and above. On the basis of the finding of a higher incidence of mammary tumours in younger animals, which are more sensitive to prolactin, it has been postulated that melengestrol acetate causes tumours indirectly in C57Han/f mice, by increasing the release of prolactin. The NOEL for induction of mammary tumours was 1 mg/kg of body weight per day.

In a study to investigate the relationship between long-term administration of melengestrol acetate, serum prolactin concentration and mammary duct proliferation, female C57Han/f mice aged 44 days were fed melengestrol acetate in the diet at concentrations providing doses equivalent to 0, 0.5, 1.5, 2.5, 5, 10 or 25 mg/kg of body weight per day for 1 year. Additional groups were also given a daily subcutaneous injection of the prolactin inhibitor 6-methyl-8β-ergoline-acetonitrile, but the dose of this compound was found to be too low and these groups were not evaluated further. Body weights were increased in animals in the highest-dose group. The serum prolactin concentration, which was determined only at termination of the study, was increased in all the treatment groups; the increase was significant in those dosed at 10 mg/kg of body weight per day and above. An increasing trend in the incidence of animals with exacerbated mammary duct proliferation was observed at doses of 2.5 mg/kg of body weight per day and above; the incidence was significantly increased at doses of 5 mg/kg of body weight per day and above. The NOEL for hormonal effects was close to 0.5 mg/kg of body weight per day.

In a follow-up study, female C57Han/f mice of 44 days of age were fed diets containing melengestrol acetate at concentrations providing doses equivalent to 0, 0.5, 1.5, 2.5, 5, 10 or 25 mg/kg of body weight per day for a maximum of about 29 months. Additional groups of animals receiving 0, 5, 10 or 25 mg/kg of body weight per day were also given a daily subcutaneous injection of 100 μg of the prolactin inhibitor 6-methyl-8β-ergoline-acetonitrile. Mice in all the treatment groups showed more rapid weight gain than controls during the first year but decreased body-weight gain during the second year. 6-Methyl-8β-ergoline-acetonitrile did not significantly affect the melengestrol acetate-induced changes in body weight. The survival rate decreased with increasing dose of melengestrol acetate, attaining significance at 5 mg/kg of body weight per day and above. Mice in which prolactin was inhibited survived significantly longer than those in matched
groups without prolactin inhibition. The only treatment-related non-neoplastic lesions that were observed consisted of increased incidences of endometrial hyperplasia, uterine adenomyosis and acute metritis (at the highest dose), and decreased numbers of cystic ovaries and cystic endometrial glands. 6-Methyl-8β-ergoline-acetonitrile did not prevent these effects. In the mammary glands of treated mice, adenocarcinomas and occasional benign adenomas were identified; a dose-related increase in the incidence of mammary tumours was observed, and the incidence of adenocarcinomas in animals dosed at 1.5 mg/kg of body weight per day and above was significantly higher than in controls. 6-Methyl-8β-ergoline-acetonitrile partially inhibited mammary tumour development in both control and melengestrol-treated groups. Examination by electron microscopy of the mammary tumours from selected animals at each dose and from controls revealed viral particles commonly associated with the murine mammary tumour virus. Melengestrol acetate decreased the incidence of ovarian tubular adenomas in animals dosed at 5 mg/kg of body weight per day and above. The incidence of hepatocellular adenomas was significantly increased in animals dosed at 5 mg/kg of body weight per day and above, including those treated with 6-methyl-8β-ergoline-acetonitrile, but the dose–response relationship was not consistent up to this dose. There was no treatment-related effect on the incidence of hepatocellular hyperplastic nodules or hepatocellular carcinoma. The Committee concluded that melengestrol acetate indirectly modulates mammary tumorigenesis in female C57Han/f mice, possibly by stimulating the secretion of prolactin. The NOEL for mammary tumorigenesis was 0.5 mg/kg of body weight per day. A NOEL could not be identified for the hormonal effects of melengestrol acetate on the ovaries and uterus. The minimally effective dose for increasing the incidence of hepatocellular adenomas was 5 mg/kg of body weight per day.

Melengestrol acetate was administered orally to male and female beagle dogs at a dose of 0, 1 or 2 μg/kg of body weight per day for 2 years, or at 8 μg/kg of body weight per day for 1 year followed by 4 μg/kg of body weight per day for another year. Females in the highest-dose groups showed clinical signs of the progestational activity of melengestrol acetate, such as pyometra and dystocia, during the second year. These animals also showed increased serum alkaline phosphatase activity and, after 18 months, an increased total leukocyte count and reduced erythrocyte count, haemoglobin concentration and erythrocyte volume fraction. Most of these changes occurred in females with abnormalities of the reproductive tract. Females in the highest-dose groups had alterations of the endome-
trium characteristic of progestational activity. No neoplastic changes were seen in the mammary gland at any dose. The Committee concluded that progestational effects were the most sensitive endpoint. The NOEL for hormonal effects was 1 µg/kg of body weight per day.

Melengestrol acetate has been tested for genotoxicity in a range of assays in vitro and in vivo. Gene mutations were not induced in *Salmonella typhimurium* or mammalian cells. Unscheduled DNA synthesis was not observed in rat primary hepatocytes or in the alkaline elution assay in Chinese hamster V79 cells. Melengestrol acetate did not induce micronuclei in the bone marrow of mice exposed in vivo by intraperitoneal injection. The Committee concluded that melengestrol acetate is not genotoxic.

In a one-generation study of reproductive toxicity in rats, melengestrol acetate was administered in the diet at concentrations equivalent to 0, 0.03, 0.06, 0.13, 0.25 or 1 mg/kg of body weight per day. Melengestrol acetate suppressed the estrus cycle at doses of 0.13 mg/kg of body weight per day and above and had significant effects on fertility and pregnancy at doses of 0.06 mg/kg of body weight per day and above: at 0.06 mg/kg of body weight per day, only one of seven dams became pregnant, whereas at 0.03 mg/kg of body weight per day all dams became pregnant. While the incidence of resorption in animals treated at 0.03 mg/kg of body weight per day and above was twice that in controls, there was no difference in litter size. The body weights of pups of treated dams during lactation were not statistically significantly different from those in the control group, although their birth weights were higher. Dams dosed at 0.06 mg/kg of body weight per day and above showed a significant increase in the serum prolactin concentration and significant decreases in the serum progesterone concentration and in the weights of the adrenals, ovaries and uterus. The histological appearance of the ovaries and uterus was consistent with progestational activity. The NOEL for reproductive toxicity was 0.03 mg/kg of body weight per day.

The effect of melengestrol acetate on reproductive performance in beagle dogs was evaluated in the 2-year study described above in which melengestrol acetate was administered orally at a dose of 0, 1 or 2 µg/kg of body weight per day, or at 8 µg/kg of body weight per day for 1 year followed by 4 µg/kg of body weight per day for another year. Animals treated at the same dose were bred, and females in the lowest-dose group were bred with males in the highest-dose group. Treatment of females was begun 120 days after estrus. Melengestrol acetate at 4 or 8 µg/kg of body weight per day suppressed estrus, but
estrus resumed within 12–201 days of cessation of treatment. Fewer females treated at the highest dose became pregnant, and parturition was impaired in animals at this dose during the second year, resulting in significantly greater pup loss. The percentage of male pups and the mean birth weight were slightly decreased at the highest dose. Melengestrol acetate did not appear to affect the fertility of male dogs. The NOEL for reproductive performance was 2 μg/kg of body weight per day.

Cows and heifers that were F₁ or F₂ progeny of melengestrol acetate-treated heifers received the drug in their diet at a concentration equal to 2 μg/kg of body weight per day for up to about 2 years, except during the breeding period. Melengestrol acetate completely suppressed estrus. The conception and pregnancy rates were not different from those of controls, except for a temporarily reduced conception rate at estrus after the last feeding of melengestrol acetate. The calves weighed less than those of controls at birth but not at weaning. At necropsy, the only treatment-related change was reduced adrenal weight. When bull calves received the same treatment for about 2 years, no adverse effect was seen on fertility, and the only effect was a reduction in adrenal weight.

In a study of developmental toxicity, pregnant rats received a single subcutaneous dose of 0, 15, 25, 50 or 100 mg/kg of body weight of a sustained-release formulation of melengestrol acetate on day 6 of gestation and were killed on day 20. Reduced litter weights and average pup weights, increased numbers of sites of fetal resorption, a larger percentage of pups with retarded development, and skeletal and visceral abnormalities were observed at doses of 25 mg/kg of body weight and above. The Committee concluded that melengestrol acetate was embryotoxic and teratogenic at these doses. A NOEL could not be identified because no information was available on the toxicokinetics of the sustained-release formulation that would allow estimation of the systemic exposure of the dams.

In another study of developmental toxicity, melengestrol acetate was administered orally to pregnant rabbits at concentrations equivalent to 0, 0.016, 0.064, 0.16, 0.4, 0.8, 1.6, 3.2 or 6.4 mg/kg of body weight per day on days 6–18 of gestation. The fetuses were removed surgically on day 28. The body weights of the does treated with doses of up to 0.16 mg/kg of body weight per day were increased, and those of animals given higher doses were slightly decreased. Melengestrol acetate was embryotoxic and fetotoxic at doses of 0.8 mg/kg of body weight per day and above, as indicated by a large increase in the numbers of sites of fetal resorption and dead fetuses. The percentage of embryonic deaths
approached 100% in the does treated at 3.2 mg/kg of body weight per day. The litter size was reduced at 1.6 mg/kg of body weight per day. The number of live fetuses and the mean litter and fetal weights were significantly lower at doses of 0.8 mg/kg of body weight per day and above. Other effects observed at 0.8 and 1.6 mg/kg of body weight per day included cleft palate, talipes, umbilical hernia and incomplete skeletal ossification. At 1.6 mg/kg of body weight per day, the male:female ratio was reduced to 0.36. The Committee concluded that the fetotoxic and teratogenic effects of melengestrol acetate in rabbits are due to its corticosteroid activity. The NOEL for embryotoxicity and teratogenicity was 0.4 mg/kg of body weight per day.

In a second study of developmental toxicity in rabbits, females received a subcutaneous injection of a sustained-release formulation of melengestrol acetate at 0, 5 or 15 mg/kg of body weight on day 6 after artificial insemination. The fetuses were delivered surgically on day 28 of gestation. At the highest dose, only one live but undersized fetus was found in the 12 litters examined. At 5 mg/kg of body weight, five live but undersized fetuses were found in the 14 litters examined. Nearly all live and dead fetuses of treated does had cleft palate. The other abnormalities observed were exencephaly, agenesis of the lens, irregular brain conformation, umbilical hernia, ablepharia, and enlarged liver. The Committee concluded that melengestrol acetate was teratogenic and embryotoxic at 15 mg/kg of body weight and fetotoxic at doses of 5 mg/kg of body weight and above. The study was not appropriate for identifying a NOEL because no information was available on the toxicokinetics of the sustained-release formulation that would allow estimation of the systemic exposure of the dams.

Observations in regularly ovulating women (number not stated) indicated that melengestrol acetate delayed the onset of menses at daily doses of 7.5 and 10 mg but not 5 mg. In three volunteers, daily doses of 2.5 mg of melengestrol acetate and 0.05 mg of ethinylestradiol suppressed endometrial proliferation. Single doses of 5, 7.5 or 10 mg of melengestrol acetate or repeated daily doses of 2.5 mg induced withdrawal bleeding in 11 estrogen-primed amenorrheic women.

The Committee concluded that the most appropriate end-point for evaluating the safety of residues of melengestrol acetate is the progestational effect in non-human primates. An ADI of 0–0.03 μg/kg of body weight was established by applying a safety factor of 200 to the minimally effective dose of 5 μg/kg of body weight per day of melengestrol acetate for effects on the menstrual cycle of cynomolgus monkeys in a study over three menstrual cycles. This safety factor was used because the ADI is not based on a clear NOEL.
Pharmacokinetic and residue data

Studies with [³H]melengestrol acetate labelled in the C6 position on the methyl group and with [¹⁴C]melengestrol acetate labelled in the C6 position on the methyl group and in the C16 position on the methylene group were conducted in young heifers. In one study, four animals received ground feed supplemented with 0.5 mg of unlabelled melengestrol acetate daily for 4 months, followed by either daily oral doses (in capsules) of approximately 0.5 mg of [³H]melengestrol acetate for 21 days (three animals) or daily oral doses (in capsules) of approximately 0.5 mg of [¹⁴C]melengestrol acetate for 7 days (one animal). The animals were killed 6 h after administration of the last capsule, and samples of their body fluids and tissues were collected and analysed for total residues. The radiolabel was eliminated in the faeces and urine in a 6:1 ratio. Independent studies of animals with bile cannulae showed that biliary excretion closely paralleled total faecal output. In the study with four animals, the highest concentration of total residues was found in liver; however, the highest percentage of parent drug was found in fat, with similar concentrations in visceral, omental and perirenal fat. The results obtained for fat and liver were similar in all four treated animals and the concentrations of melengestrol acetate residues found in muscle were all close to or below the limit of quantification of the analytical method used (Table 4). Individual metabolites were not identified because they occurred at such low concentrations.

In another study, in which 4.0 mg of [³H]melengestrol acetate was administered orally daily for 15 days to three Holstein heifers to achieve a steady-state concentration of the drug, 83% (standard deviation, 13%) of the daily dose was recovered in urine and faeces on the same day. The animals were killed 1, 4 and 10 days after the last dose, and the total residues in selected edible tissues were determined. The results confirmed that the concentrations of total residues in perirenal, visceral and omental fat were similar and decreased at similar rates. Although the dose was eight times the recommended dose, no residues were found in muscle.

In order to estimate the concentrations of residues of melengestrol acetate in edible tissues, one heifer was treated with 1000 mg orally (corresponding to 2000 times the highest recommended dose) for 5 days, and then received 500 mg by subcutaneous injection and another 500 mg intramuscularly on the fifth day. The animal was killed on the sixth day, and samples of the edible tissues were collected and analysed for residues of the parent drug. The residue concentrations were highest in fat (3300 µg/kg), followed by liver (880 µg/kg), muscle (220 µg/kg) and kidney (120 µg/kg). These results are consistent with
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Concentration of total residues (µg/kg)</th>
<th>Percentage of total residues accounted for by parent drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>0.6</td>
<td>31&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>&lt;LOQ</td>
<td>45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>12</td>
<td>30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.7</td>
<td>24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>130&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat (perirenal)</td>
<td>7.5</td>
<td>78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>86&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

LOQ: limit of quantification (0.5 µg/kg).

<sup>a</sup> Animals received unlabelled melengestrol acetate in their feed at 0.5 mg daily for 4 months, followed by either <sup>3</sup>Hmelengestrol acetate orally at 0.5 mg/day for 21 days or
<sup>b</sup> <sup>14</sup>Cmelengestrol acetate orally at 0.5 mg/day for 7 days.

<sup>b</sup> <sup>3</sup>HMelengestrol acetate.

<sup>a</sup> <sup>14</sup>CMelengestrol acetate.

the lack of detectable residues in muscle reported in the studies in which the animals were treated at the recommended doses.

The Committee reviewed a large number of residue-depletion studies of melengestrol acetate. In one pivotal study, 174 samples of perirenal fat were obtained from heifers in 25 feedlots which had been given a conventional solid supplement containing melengestrol acetate at the highest recommended dose of 0.5 mg/animal per day. Another 84 fat samples were obtained from heifers in 12 other feedlots which had received a dose of 0.5 mg/animal per day of a liquid formulation delivered into the complete feed. Of the 37 groups of cattle, 27 were slaughtered less than 10 h after the last treatment, eight were slaughtered between 11 h and 16 h after the last treatment, and two were slaughtered 18 and 27.5 h after the last treatment. The concentrations of residues of melengestrol acetate in samples of fat from animals treated with the liquid formulation were significantly lower than those in samples from animals given the solid formulation. The 99th
percentile concentration of residues of melengestrol acetate in fat samples from all animals was about 18µg/kg, and the median concentration was about 6µg/kg.

A relationship was established between the administered dose and the concentrations of residues of melengestrol acetate found during treatment on the basis of the results of several similar studies conducted with doses ranging from 0.3 to 10mg/animal per day. This relationship was used to estimate that the 99th percentile concentration of residues of parent melengestrol acetate in fat would be about 10µg/kg when the approved dose of 0.25mg/animal per day was administered.

Analytical methods
The available analytical methods were developed before the introduction of GLP. Validation of the method used in the studies summarized by the Committee was based mainly on the analytical recovery of melengestrol acetate. The procedure involves several solvent partition and chromatographic separation and purification steps, followed by gas–liquid chromatography (GLC) and detection by electron capture. In a collaborative study in seven laboratories in 1975, the method was tested on samples of muscle, liver, kidney and fat of cattle. Its accuracy and recovery were considered to be satisfactory. The authors concluded that the method could be used to distinguish samples containing residues at a concentration of 10µg/kg from samples that did not contain residues of melengestrol acetate. However, it was not suitable for differentiating samples containing residues at concentrations of 10µg/kg and 20µg/kg.

This collaborative study resulted in Official Method 976.36 of the Association of Official Analytical Chemists (AOAC International), which is applicable for the analysis of residues of melengestrol acetate in animal tissues (muscle, liver, kidney and fat) at concentrations down to 10µg/kg when a calibration curve that includes concentrations of 10 and 30µg/kg is used. However, the method requires large volumes of organic solvents, including benzene, use of which is prohibited in many parts of the world because of its carcinogenicity.

A GLC–MS procedure can be used to confirm the results for fat samples containing melengestrol acetate at about 10µg/kg. The characteristic mass ions of melengestrol acetate are m/z 311, 321, 336, 337 and 354 atomic mass units.

Choice of marker residue and target tissue
If the usual food factors are applied, liver would be the main dietary source of residues of melengestrol acetate. However, liver and fat are
equally important sources of residues. The contributions of residues in muscle and kidney are minor. Of the four standard edible tissues, fat contains the highest concentrations of melengestrol acetate. In studies in which radiolabelled melengestrol acetate was administered to cattle, the parent drug accounted for 33% (range, 28–37%) of the total residues in liver and 85% (range, 75–94%) of those in fat. Therefore, melengestrol acetate and fat are appropriate as the marker residue and target tissue, respectively.

**Maximum Residue Limits**

In reaching its decision on MRLs for melengestrol acetate, the Committee took the following factors into account:

- An ADI of 0–0.03 μg/kg of body weight was established, which is equivalent to a maximum ADI of 1.8 μg for a 60-kg person.
- The available analytical methods date from 1968–76 and do not meet current standards for regulatory use. The Committee noted that analytical instrumentation and data processing for use in residue analysis have improved dramatically since that time.
- The sponsors did not submit an analytical method suitable for monitoring purposes. A variety of potentially suitable, modern methods for regulatory purposes, which have been validated in a single laboratory and are cost-effective and efficient, are described in the literature.
- Only fat and liver contain concentrations of the marker residue that are quantifiable on a routine basis; methods with limits of quantification greater than 0.3 μg/kg are unlikely to permit quantification of residues in muscle from animals treated with recommended doses of melengestrol acetate.
- The steady-state concentrations of melengestrol acetate residues in muscle and kidney are about 0.7 and 1.6 μg/kg respectively, with the parent drug accounting for approximately 45% of the total residues in both tissues.
- As inadequate information was available on the structure and activity of metabolites of melengestrol acetate, they were assumed to be as progestogenic as the parent drug and were taken into account by the Committee in recommending MRLs.

The Committee recommended temporary MRLs for melengestrol acetate of 2 μg/kg for liver and 5 μg/kg for fat in cattle, expressed as parent drug. MRLs were not recommended for muscle and kidney as the concentrations of residues in these tissues are generally low and, in the case of muscle, often at or below the limit of quantification of the analytical method. The MRLs were made temporary, pending the
Table 5
Theoretical maximum daily intake of melengestrol acetate residues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Recommended MRL (µg/kg)</th>
<th>Estimate of total residues (µg/kg)</th>
<th>Theoretical maximum daily intake (µg melengestrol acetate equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>6</td>
<td>0.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Expressed as parent drug.
* Based on a daily intake of 300 g of muscle, 100 g of liver, and 50 g each of kidney and fat.
* MRLs were not recommended for muscle and kidney.
* Melengestrol acetate accounted for 33% of the total residues in liver.
* Melengestrol acetate accounted for 85% of the total residues in fat.

receipt of information on an analytical method suitable for quantifying residues of melengestrol acetate in liver and fat tissue. This information is required for evaluation in 2002.

From these MRLs, the theoretical maximum daily intake of residues would be 0.9 µg (see Table 5).

4. **Future work**

The Committee noted several issues that might be addressed in relation to its risk assessment procedures on the basis of a preliminary review of the document on *Procedures for recommending Maximum Residue Limits — residues of veterinary drugs in food (1987–1999)* (7). These issues should be addressed during the process of updating the principles of assessment, as recommended at the *Conference on International Food Trade Beyond the Year 2000* (1).

5. **Recommendations**

1. Recommendations relating to specific veterinary drugs, including ADIs and MRLs, are given in section 3 and Annex 2.
2. In view of the continuing need for evaluations of veterinary drugs, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held regularly for this purpose.
Acknowledgement

The Committee wishes to thank Mrs. E. Heseltine, Communication in Science, Lajarthes, Saint-Léon-sur-Vezère, France, for her assistance in the preparation of the report.

References


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


12. Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers,


63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.
64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.
82. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41, 1988 (out of print).

1 The full text is available electronically on the Internet at http://www.who.int/pcs.


121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 418, 1996.


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

Anthelminthic agent
Ivermectin

ADI: 0–1 μg/kg of body weight (established at the fortieth meeting of the Committee (WHO Technical Report Series, No. 832, 1993)).

Residue definition: 22,23-Dihydroavermectin B₁₅.

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td></td>
<td>100⁴</td>
<td></td>
<td>40⁴</td>
<td>10⁵</td>
</tr>
<tr>
<td>Other species</td>
<td></td>
<td>15⁵</td>
<td></td>
<td>20⁵</td>
<td></td>
</tr>
</tbody>
</table>

⁴ These MRLs were recommended at the fortieth meeting of the Committee (WHO Technical Report Series, No. 832, 1993).
⁵ Temporary MRL, pending the receipt of the full set of data for validation of the analytical method and information on other routes of application of ivermectin to cattle. This information is required for evaluation in 2002.
⁶ These MRLs were recommended at the thirty-sixth meeting of the Committee (WHO Technical Report Series, No. 799, 1990).

Antimicrobial agents
Flumequine

ADI: 0–30 μg/kg of body weight (established at the forty-eighth meeting of the Committee (WHO Technical Report Series, No. 879, 1998)).

Residue definition: Flumequine.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>500</td>
</tr>
<tr>
<td>Pigs</td>
<td>500</td>
</tr>
<tr>
<td>Sheep</td>
<td>500</td>
</tr>
<tr>
<td>Chickens</td>
<td>500</td>
</tr>
<tr>
<td>Trout</td>
<td>500⁶</td>
</tr>
</tbody>
</table>

⁶ Muscle/skin in normal proportions.
Lincomycin

ADI: 0–30 µg/kg of body weight.

Residue definition: Lincomycin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Cattle</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pigs</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>Sheep</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chickens</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Temporary MRLs, pending the receipt of data from residue-depletion studies in cattle, sheep and chickens, which show that lincomycin is the major microbiologically active residue in the edible tissues. These data are required for evaluation in 2002.

<sup>b</sup> Before recommending an MRL for chickens’ eggs, the Committee would wish to see the following:

— data from residue-depletion studies showing that lincomycin is the major microbiologically active residue in eggs;
— the results of a residue-depletion study in which the GC–MS method is used to analyse residues in eggs.

This information is required for evaluation in 2002.

Oxytetracycline

ADI: 0–30 µg/kg of body weight (group ADI for tetracycline, oxytetracycline and chlortetracycline; established at the fiftieth meeting of the Committee (WHO Technical Report Series, No. 888, 1999)).

Residue definition: Oxytetracycline, alone or in combination with chlortetracycline and tetracycline.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRL (µg/kg)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>Cattle</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>600&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pigs</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>600&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sheep</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>600&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Poultry</td>
<td>200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>600&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fish</td>
<td>200&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Giant tiger prawn (Penaeus monodon)</td>
<td>200&lt;sup&gt;a,d&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> These MRLs were recommended at the fiftieth meeting of the Committee (WHO Technical Report Series, No. 888, 1999).

<sup>b</sup> At its forty-seventh meeting (WHO Technical Report Series, No. 876, 1998), the Committee recommended that the MRL for oxytetracycline in fat be withdrawn, and decided that the MRLs for chlortetracycline and tetracycline in fat were not required.

<sup>c</sup> Temporary MRL, pending the results of a residue-depletion study and information on the validated analytical method for fish. These data are required for evaluation in 2002.

<sup>d</sup> Applies only to oxytetracycline.
**Tilmicosin**

**ADI:** 0–40μg/kg of body weight (established at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

**Residue definition:** Tilmicosin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>100(^a)</td>
<td>1000(^a)</td>
<td>300(^a)</td>
<td>100(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Pigs</td>
<td>100(^a)</td>
<td>1500(^a)</td>
<td>1000(^a)</td>
<td>100(^a)</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>100(^a)</td>
<td>1000(^a)</td>
<td>300(^a)</td>
<td>100(^a)</td>
<td>—(^b)</td>
</tr>
</tbody>
</table>

\(^a\) These MRLs were recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998).

\(^b\) The temporary MRL recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998) was not extended, as the results of the requested study with radio-labelled tilmicosin in lactating sheep, to determine the relationship between total residues and the parent drug in milk, were not available.

**Insecticides**

**Cyhalothrin**

**ADI:** 0–2μg/kg of body weight (designated as temporary, pending the results of studies appropriate for identifying a NOEL for neurobehavioural effects in laboratory animals; these results are required for evaluation in 2002).

**Residue definition:** Sum of the concentrations of the isomers of cyhalothrin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg),(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>20</td>
</tr>
<tr>
<td>Pigs</td>
<td>20</td>
</tr>
<tr>
<td>Sheep</td>
<td>20(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Temporary MRLs.

\(^b\) Data on the validation of the analytical method for sheep liver, to confirm the limit of quantification of 10μg/kg, are required for evaluation in 2002.

**Cypermethrin**

**ADI:** 0–50μg/kg of body weight (established at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

**Residue definition:** Sum of the concentrations of the isomers of cypermethrin.
<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Kidney</td>
<td>Fat</td>
<td>Milk</td>
</tr>
<tr>
<td>Cattle</td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
</tr>
<tr>
<td>Sheep</td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
</tr>
<tr>
<td>Chickens</td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
</tr>
</tbody>
</table>

* The temporary MRLs recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998) were not extended, as the requested information was not provided and there was no indication that it would be provided in the future.

**α-Cypermethrin**

**ADI:** 0–20µg/kg of body weight (established at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998)).

**Residue definition:** Sum of the concentrations of the isomers of α-cypermethrin.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Kidney</td>
<td>Fat</td>
<td>Milk</td>
</tr>
<tr>
<td>Cattle</td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
</tr>
<tr>
<td>Sheep</td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
</tr>
<tr>
<td>Chickens</td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
<td><em>a</em></td>
</tr>
</tbody>
</table>

* The temporary MRLs recommended at the forty-seventh meeting of the Committee (WHO Technical Report Series, No. 876, 1998) were not extended, as the requested information was not provided and there was no indication that it would be provided in the future.

**Dicyclanil**

**ADI:** 0–7µg/kg of body weight.

**Residue definition:** Dicyclanil.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (µg/kg)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Sheep</td>
<td>200</td>
<td>400</td>
<td>400</td>
</tr>
</tbody>
</table>

**Permethrin**

pesticide)). The Committee was unable to establish an ADI for the 80:20 cis:trans isomeric mixture proposed for use as a veterinary drug because of the lack of information on toxicity.

**Residue definition:** Sum of the concentrations of the isomers of permethrin. The Committee was unable to recommend MRLs for the 80:20 cis:trans isomeric mixture of permethrin in the absence of an ADI.

**Metrifonate (trichlorfon)**

**ADI:** 0–20μg/kg of body weight.

**Residue definition:** Metrifonate.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>50³</td>
</tr>
</tbody>
</table>

³ The Committee noted that the concentrations of residues were very low in muscle, liver, kidney and fat. These MRLs are for guidance only and are based on the limit of quantification of the analytical method.

**Production aid**

**Melengestrol acetate**

**ADI:** 0–0.03μg/kg of body weight.

**Residue definition:** Melengestrol acetate.

<table>
<thead>
<tr>
<th>Species</th>
<th>Recommended MRLs (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
</tr>
<tr>
<td>Cattle</td>
<td>—³</td>
</tr>
</tbody>
</table>

⁻ MRLs were not recommended for muscle or kidney as the concentrations of residues were below the limit of quantification of the analytical method.

⁻ Temporary MRL, pending the receipt of information on an analytical method suitable for quantifying residues of melengestrol acetate in liver and fat tissue. This information is required for evaluation in 2002.

² An MRL was not recommended for milk as the drug is not used in dairy cows.
Annex 3

Proposed draft definitions of commodities for Volume 3 of Residues of veterinary drugs in foods¹

Meat

Proposed draft definition
The skeletal muscular tissue of an animal carcass or cuts of such tissue from an animal carcass. It includes interstitial and intramuscular fat. It may also include bone, connective tissue and tendons, as well as nerves and lymph nodes in natural proportions. It does not include edible offal or trimmable fat.

Portion of the commodity to which the MRL applies
The whole commodity without bones.

Current definition
The edible part of any mammal.

Muscle and fat

Proposed draft definitions

Muscle
Muscle tissue only (definition established and adopted by the Joint FAO/WHO Expert Committee on Food Additives).

Fat
The lipid-based tissue that is trimmable from an animal carcass or cuts from an animal carcass. It may include subcutaneous, omental or perirenal fat. It does not include interstitial or intramuscular carcass fat or milk fat.

Portion of the commodity to which the MRLs apply
The whole commodity. For fat-soluble compounds, the MRL applies to the trimmable fat. For those compounds where the trimmable fat is insufficient to provide a suitable test sample, the whole commodity (muscle and fat but without bone) is analysed and the MRL applies to the whole commodity (e.g. rabbit meat).

Current definitions
None.