EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Thirty-eighth report of the 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:

Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 29, in press.

Residues monographs are issued separately by FAO under the title:

Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/4, in press.

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

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

The 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 22 to 31 January 1991. The meeting was opened by Mr H.S. Dhillon, Director, Division of Health Education, on behalf of the Directors-General of the World Health Organization and the Food and Agriculture Organization of the United Nations.

Mr Dhillon noted that the meeting was the fourth that the Committee had devoted exclusively to the evaluation of veterinary drug residues. To date, more than 20 veterinary drugs had been evaluated and extensive methodology for the safety assessment of residues of veterinary drugs in food developed by the Committee. It was extremely important to users of the evaluations that reports maintain consistency and explain clearly the basis for decisions taken.

Mr Dhillon also noted that the first intergovernmental conference since 1973 to consider issues related to chemicals in food – the Joint FAO/WHO Conference on Food Standards, Chemicals in Food, and Food Trade – was to be held in Rome in March 1991. One of the items for discussion would be residues of veterinary drugs in food. Both WHO and FAO attached a great deal of importance to the Conference, and its recommendations were expected to provide guidelines for future work in this area.

The three previous meetings of the Committee to consider veterinary drug residues in food (Annex 1, references 80, 85 and 97) had been held in response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (1). The present meeting was convened in response to the recommendation made at the thirty-sixth meeting of the Committee that meetings on this subject should be held regularly (Annex 1, reference 97). 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 of animal origin. The specific tasks before the Committee were:

(a) to further elaborate principles for evaluating the safety of residues of veterinary drugs in foods and for determining acceptable and safe levels for such residues when the drugs in question are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (see section 2);

(b) to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annexes 2 and 3); and

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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 37 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
(c) to discuss matters of interest arising from the report of the Fifth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (see Annex 4) (2).

2. **General considerations**

2.1 **Principles governing the safety evaluation of residues of veterinary drugs in food**

In making recommendations on the safety of residues of veterinary drugs in food, the Committee took into consideration the principles contained in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76), in the thirty-second, thirty-fourth, and thirty-sixth reports of the Committee (Annex 1, references 80, 85, and 91), and in the report of the Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Food (1).

2.2 **Safety factors**

In calculating the Acceptable Daily Intake (ADI) for a veterinary drug, the Committee has usually followed the procedures described in *Principles for the safety assessment of food additives and contaminants in food* (Annex 1, reference 76) and applied a safety factor to the no-observed-effect level (NOEL) derived from the most appropriate study. This safety factor usually has a value of 100 in the case of a NOEL derived from a long-term animal study, on the assumption that humans are ten times as sensitive as the test animal used and that there is a ten-fold range of sensitivity within the human population. When no adverse effects are seen in long-term studies, a safety factor of 100 may be applied to the NOEL derived from short-term studies where higher dose levels have been used and an effect has been noted (e.g., a 3-month study). However, there are times when a safety factor of 100 is considered insufficient. Thus higher safety factors may be required when the data are incomplete, when the study in which the NOEL was established was inadequate (e.g., too few animals, no individual animal results), or when irreversible, and especially teratogenic and carcinogenic, effects have been seen. The higher safety factors normally used by the Committee are 200, 500, 1000 and 2000. Safety factors are not usually appropriate for genotoxic carcinogens. Where the only effects of note are seen in human studies, a lower safety factor, usually ten, may be applied.

It was recognized that there might be occasions when drug metabolites present as residues were devoid of the specific activity possessed by the parent drug that gave rise to major concern. In such cases, the parent drug's activity would be discounted in establishing an ADI on which to base the Maximum Residue Limit (MRL); the ADI would instead be based on a toxicological property of the metabolites with an appropriate safety factor applied.
The Committee stressed that, in all circumstances, each drug would be assessed on its own merits.

2.3 Consideration of pharmacological effects in establishing ADIs

The Committee discussed the relevance of pharmacological activity in the safety assessment of residues of veterinary drugs. It recognized that a desired pharmacological effect in animals or humans might well be unwanted in consumers of residues of a pharmacologically active agent, particularly if they were unduly sensitive to such an effect. The β-adrenoceptor-blocking agents such as carazolol provided good examples of drugs whose inadvertent pharmacological effect could be extremely undesirable in individuals with cardiovascular or respiratory disease. Other examples would include tranquillizers, β-adrenoceptor agonists, vasodilators, and anaesthetics. Consequently, major pharmacological effects should be regarded as adverse effects and should be assessed together with the toxicological effects of veterinary drug residues. If there are no toxicological data of overriding importance or if the pharmacological effects are the most relevant and sensitive, the ADI should be determined on the basis of pharmacology. In animal studies, the NOEL derived from the results of the most relevant (e.g., by the oral route) and most sensitive pharmacological tests should be chosen for use in establishing the ADI. However, data on humans would be preferable if available and a lower safety factor (e.g., ten) could then be contemplated. Where possible, data on sensitive populations should be obtained. The Committee considered that a case-by-case approach was the most appropriate for the evaluation of pharmacologically active compounds; many exert a variety of effects, and the nature of these effects and the relevance of effects in animals to possible adverse effects in humans would need to be considered.

2.4 Pharmacologically active residues of veterinary drugs

The degree of concern over the presence of xenobiotics in food is influenced by many factors. Of particular relevance to the Committee are residues of drugs used as animal medication or as aids to animal production, and the frequency with which the consumer might be exposed to them. A drug whose toxicity profile was unsatisfactory and that was used widely and for lengthy periods during food-animal production would cause the highest level of concern. Human exposure to such a drug in food could be expected to occur widely and repeatedly.

Also of concern to the Committee would be a drug whose presence in food could result in a pharmacological effect in the consumer in the absence of conventional toxicological effects. This would constitute an intrusion into the body's homeostasis, against which consumers could reasonably expect to be protected. Drugs still present in the animal at the time of slaughter and at a concentration compatible with a pharmacological effect pose the greatest risk in this respect.
Azaperone, the phenothiazine tranquilizers chlorpromazine and propionylpromazine, and the β-adrenoceptor-blocking agent carazolol, all described in the present report, fall into this category. The Committee was specifically aware of their use in pigs in the immediate pre-slaughter period. In particular, chlorpromazine is known to persist for very long periods in the human body, and it is therefore possible that repeated intake could result in accumulation of the drug, thereby increasing the pharmacological response.

The continued use in animals of any drug capable of leaving residues likely to be ingested by humans in food is permissible only when that use is shown to be acceptably safe. Conventionally, safety is considered to be established when the residue is shown not to produce any toxic action in humans despite its daily ingestion over a lifetime. Given that the protective effect of drugs used to minimize pre-slaughter deaths is required right up to the time of slaughter, it is likely that such drugs will be present in animal tissues in concentrations higher than those of drugs used earlier in the production process. Acceptable safety for the consumer would in these circumstances require the demonstration not only that such residues are toxicologically acceptable but also that no pharmacological effect is produced. In the case of a drug allocated a zero withdrawal period (such as azaperone in some countries), it is unlikely that these criteria would be met. Where a drug is licensed for use with a longer withdrawal period (for propionylpromazine, for example, it is usually 5 days), improper use is more likely and the criteria are even less likely to be met.

These considerations have caused some manufacturers to remove immediate pre-slaughter use from the clinical indications for their products. The Committee took the view that this use should not be allowed unless specific studies show it to be without unacceptable toxicological or pharmacological risk for the consumer, if account is taken not only of the concentrations present throughout the tissues but also and more particularly of the unabsorbed residue of the administered dose of the drug. This latter issue is further discussed in section 2.5.

2.5 Residues at the injection site

In assessing the contribution of drug residues in edible tissues to the total daily intake, the Committee does not include residues that persist at or near the injection site of the drug. None the less, the Committee is concerned about the possible high levels of residues at injection sites and the fact that such sites may be in non-discarded edible tissues.

The Committee considered the following four circumstances of use of drugs by parenteral administration or implantation:

1. Use of an allowed drug in accordance with approved specifications.
2. Use of an allowed drug in a manner not in accordance with approved specifications by virtue of:
- administration at an unapproved site;
- administration to an unapproved species;
- administration for an unapproved indication.
3. Improper use of an allowed drug by virtue of failure to observe the prescribed withdrawal times.
4. Use of a prohibited substance.

The Committee must always consider the first circumstance in its safety evaluations of veterinary drug residues and, if it is to assess the implications of residues at the injection site, requires information with respect to drug dose, formulation and time elapsed since injection. The second and third circumstances are of concern only in that the Committee would wish to draw attention to the additional residue burden for the consumer that might result from these uses of an allowed drug.

Examples of types of use that may cause high levels of residues at the injection site include:

- Administration of a drug immediately before slaughter, as when pigs are given tranquillizing drugs by injection immediately before transport to slaughter (this indication requires the full pharmacological effect of the drug to be exerted right up to the moment of slaughter and renders a withdrawal period impossible). The possibility of a pharmacological effect in the consumer is then of concern in addition to the more usual requirement of freedom from conventional toxicity.
- Treatment of animals slaughtered for human consumption before appropriate withdrawal times are observed (casualty slaughter).
- Implantation or injection of long-acting drugs with slow rates of absorption, e.g., anabolic agents, in edible tissues.

Such problems could be avoided by ensuring that the injection site is excised and discarded at slaughter. In practice, this is difficult to achieve because the site is not readily recognizable unless the drug is coloured or implanted in an inedible tissue. The site, its dimensions, and the efficiency of its removal would be liable to considerable variation. To accommodate these variables, a relatively large amount of tissue would have to be discarded, and it is probable that this practice would be seen as uneconomic.

In order to prevent improper practices, the Committee may recommend specific restrictions on the use of a drug in particular situations.

The fourth circumstance is not of direct concern to the Committee and is regarded as the responsibility of individual countries through their national control programmes.
2.6 Relationship of withdrawal time to recommended Maximum Residue Limits

In assessing the safety of veterinary drug residues, the Committee determines the MRL expressed in terms of a named marker residue for target tissues of interest. The Committee does not attempt to derive, from the residue kinetics, the appropriate withdrawal times to be observed in order to ensure that the concentration of residues in food will be below the established MRL. In fact, residue kinetics and withdrawal times depend on various parameters strictly linked to a given veterinary drug, including, but not limited to, the pharmaceutical formulation, the concentration of the active ingredient, the dosage, and the route of administration. The determination of the appropriate withdrawal time for a given veterinary drug in order to comply with an assigned MRL is the responsibility of the appropriate national licensing authority. Nevertheless, when determining MRLs, the Committee verifies that those that it recommends can be achieved through realistic withdrawal times and established good practices in the use of veterinary drugs.

The “withdrawal time”, as usually defined by national licensing authorities, is the interval between the time of the last administration of a veterinary drug and the time when the animal can be safely slaughtered for food, or the milk or eggs can be safely consumed. After an MRL has been established for a named marker residue, the corresponding withdrawal time must be calculated such that the concentration of this residue in the target tissue falls with reasonable statistical certainty below it.

A withdrawal time should therefore be established on the basis of a statistical tolerance limit providing an interval within which a given percentile of the population of residue data lies, with a given level of confidence. The Committee agreed to use the 99th percentile with a 95% confidence level for verifying that recommended MRLs can be achieved through realistic withdrawal times, since it is known that a withdrawal period based on mean values of residue data may result in failure of a considerable number of samples in a national residue-monitoring programme to meet the requirements.

2.7 Naming of animal species and other parameters in the recommendation of Maximum Residue Limits

Only the target tissue (or milk) and the marker residue, where applicable, were indicated for the MRLs recommended at the thirty-fourth meeting of the Committee; the animal species were not named (Annex 1, reference 85). This oversight was corrected at the thirty-sixth meeting, where animal species were named as well as target tissue and marker residues (Annex 1, reference 97). At that meeting, however, the term “all species” was used to indicate all animal species for which data had been submitted. It was later decided by the Committee that that was too general a statement and that it would be preferable to name the specific animal species in all cases.
At the present meeting, the Committee decided that the following
requirements would be satisfied in the recommendation of MRLs for
veterinary drugs:

• All animal species would be named individually.
• Target tissues (liver, kidney, muscle, or fat) or food products obtained
  from treated animals (milk or eggs) would be identified.
• The general term “edible tissues” would be used when the MRL
  referred to all edible tissues (liver, kidney, muscle, and fat) of the named
  animal species.
• Marker residues would be identified where appropriate. Where no
  marker residue was named, it would be assumed that the MRL had
  been established on the basis of the parent drug.

The Committee also decided that, for edible tissues, at least two target
 tissues would be identified whenever possible, one being muscle or fat and
 the other liver or kidney. Selection of an appropriate target tissue permits
 regulation of the MRL in international trade in meat as well as in national
 control programmes.

In recommending MRLs in specific species, the Committee did not wish to
 preclude the occasional and limited prescribed therapeutic use of drugs in
 unnamed species that should not give rise to residues in food of animal
 origin. However, for any widespread or recommended new uses in food
 animals, sufficient information about metabolism and residues would be
 required to permit the establishment of an MRL.

3. Comments on residues of specific veterinary
drugs

The Committee evaluated for the first time the safety and residues of one
β-adrenoceptor-blocking agent, three anthelmintic agents, and three
tranquillizers. It re-evaluated the safety and residues of three antimicrobial
agents. The recommendations made with regard to the compounds on the
agenda are given in Annex 2, while details of further toxicological studies
and other information required or desired are given in Annex 3.

Toxicological monographs were prepared for all the substances
summarized in this section except sulfadimidine. Residues monographs
were prepared for all of them except sulfadimidine and chlorpromazine.

3.1 β-Adrenoceptor-blocking agent

3.1.1 Carazolol

Carazolol had not been previously reviewed by the Committee.

The compound is a nonspecific β-adrenoceptor-blocking agent, primarily
used in pigs to prevent sudden death due to stress during transport.
Toxicological data

The Committee considered pharmacological and toxicological data from pharmacodynamic, pharmacokinetic, metabolism, acute and short-term toxicity, carcinogenicity, genotoxicity, reproduction, and teratology studies, as well as the results of clinical trials in humans.

The distribution, excretion and biotransformation of radiolabelled carazolol were studied in rats, rabbits and dogs. After oral ingestion, carazolol was rapidly absorbed. In rats, the radioactivity was widely distributed in the tissues, with the highest levels in the liver, kidneys, and lungs. Excretion was almost complete within 48 hours, equally divided between the urine and faeces. In rabbits, peak plasma levels were reached 1 hour after a single oral dose of 10 mg per kg of body weight, and about 60% of the dose was excreted in urine. Following oral administration of carazolol in dogs, 13% of the radioactivity was excreted in urine and 45% in faeces within 48 hours. In the urine of a dog given 10 mg of $[^{14}C]$carazolol per kg of body weight intravenously, the parent compound and six metabolites were identified. Both carazolol and its glucuronide, lactate, and acetate metabolites have been identified in the urine of pigs and humans.

In a short-term study in rats, in which carazolol was administered in the diet at levels up to 400 mg/kg of feed for 13 weeks, females exhibited a decrease in both body weight and food intake at the highest dose. Heart rate was decreased even at the lowest dose of 40 mg/kg of feed. When rats received carazolol in the feed for 1 year, a decrease in body weight due to decreased food intake was observed at the highest dose level in both males and females only after progressively increasing this dose to 1200 and subsequently to 1800 mg/kg of feed. A statistically significant increase in relative heart weight was observed in rats at all dose levels, but this was not dose-related.

In short-term studies in dogs dosed orally at up to 20 mg per kg of body weight per day, blood chemistry and histopathological changes provided evidence of hepatotoxicity. The NOEL was 3 mg per kg of body weight per day. In a 1-year study, changes in relative organ weights (liver, kidney and testis) were observed at the dose of 30-60 mg per kg of body weight per day, with a NOEL of 15 mg per kg of body weight per day.

In a long-term study with rats at dose levels of 100, 300, and 900 mg/kg of feed, body-weight gain was reduced, even at the lowest dose level in females, and haematological and biochemical changes were found at the two highest dose levels. No increase in tumour incidence was observed.

In teratogenicity studies in rats, no malformations were observed at doses of up to 100 mg per kg of body weight per day but embryotoxicity and maternal toxicity were observed at doses of 30 mg per kg of body weight per day and above. The NOEL was 15 mg per kg of body weight per day. In one of three teratogenicity studies in rabbits with 13 or 14 mothers per
dose group, one fetus with structural malformations was observed in the mid-dose group and one in the high-dose group, giving a NOEL of 6.25 mg per kg of body weight per day.

In a two-generation reproduction study in rats, in which carazolol was administered by gavage at doses of up to 60 mg per kg of body weight per day, an increase in post-implantation losses and a decrease in the number of live pups in the first and second generations were observed in both the mid- and the high-dose groups. The NOEL in this study was 15 mg per kg of body weight per day.

Carazolol gave negative results in five in vitro and in vivo genotoxicity tests.

The β-adrenoceptor-blocking activity of carazolol was established in specific function tests in mice, rats, rabbits, and dogs. A NOEL of 0.02 mg per kg of body weight for the inhibition of isoprenaline-induced tachycardia was observed in rabbits after administration of a single oral dose.

In a clinical trial with healthy human volunteers, the effect on cardiac function was determined following administration of a single oral dose of 5 or 7 mg of carazolol per person. From the dose-response curve, a dose without effect of about 0.01 mg per kg of body weight was extrapolated. Patients suffering from chronic bronchitis showed a clear effect on respiratory function after a single oral dose of 0.7 mg per person and a marginal effect at a dose of 0.1 mg per person. Based on these results, a NOEL of about 0.03 mg per person, equivalent to 0.5 μg per kg of body weight, was extrapolated, but the Committee noted that no information was provided about the procedures used in these extrapolations.

The Committee noted that most of the toxicological data were available only in summary form and that only one long-term toxicity/carcinogenicity study was available. Since the NOEL of 3 mg per kg of body weight per day, observed in short-term toxicity studies in dogs, was several orders of magnitude higher than the NOELs for carazolol in pharmacological function studies, the Committee concluded that the pharmacological effect provided a more appropriate basis for the safety evaluation of residues. Because the information required to extrapolate to a dose without effect was lacking in the human studies, the Committee was not able to derive a clear NOEL for humans that would also cover specific groups who might be at risk, such as people suffering from cardiac or respiratory disease, particularly asthma. A temporary ADI of 0-0.1 μg per kg of body weight was therefore established, based on a NOEL of 0.02 mg per kg of body weight for the inhibition of isoprenaline-induced tachycardia in rabbits and the application of a conventional safety factor of 200 for a temporary ADI based on animal studies. The Committee noted that, if the pharmacological NOELs extrapolated from the human studies were used, an ADI of a similar order of magnitude would be obtained.
Table 1
Total residues (μg carazolol equivalents per kg) of [14 C]carazolol in pigs

<table>
<thead>
<tr>
<th>Tissue or site</th>
<th>Withdrawal time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2^a</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.0</td>
</tr>
<tr>
<td>Liver</td>
<td>33.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>20.9</td>
</tr>
<tr>
<td>Fat</td>
<td>4.3</td>
</tr>
<tr>
<td>Injection site</td>
<td>10.2</td>
</tr>
<tr>
<td>Brain</td>
<td>1.6</td>
</tr>
<tr>
<td>Lungs</td>
<td>49.3</td>
</tr>
</tbody>
</table>

^a First study.
^b Second study.

Residue data
Little information was available on the metabolism of carazolol in animals. Limited information was provided on three metabolites, carazolol glucuronide, carazolol lactate, and carazolol acetate, in the urine of pigs and humans, but there was no information on metabolism in cattle or in laboratory species other than dogs.

Results were available from residue-depletion studies in pigs and cattle.

Pigs. [14 C]Carazolol was administered intramuscularly behind one ear at a dose of 10 μg per kg of body weight to ten castrated male pigs weighing 40-50 kg. Total residues, as equivalents of carazolol, were determined in the tissues of pigs slaughtered 2, 8, and 16 hours after dosing.

In a second study, [14 C]carazolol was administered intramuscularly behind one ear at a dose of 10 μg per kg of body weight to three castrated male pigs weighing 63 kg. Total residues, as equivalents of carazolol, were determined in the tissues of pigs slaughtered 4 and 6 hours after dosing.

The dose of carazolol used in the two radiometric studies was the same, and the results were therefore combined, as shown in Table 1.

While the radiometric studies provided information on the total residues, they gave no indication of the concentrations of either the parent drug or individual metabolites.

A second set of data was obtained by means of high-performance liquid chromatography (HPLC) on the residues of parent drug in the tissues of one pig; no estimate of total residues was possible in this study. A castrated male pig weighing 80 kg was injected intramuscularly with carazolol at a dose of 10 μg per kg of body weight. Samples were collected 2 hours after injection from six sites in the liver, three in the kidney and two in the fillet, and the residues of carazolol determined by HPLC with fluorescence detection and a limit of detection of 0.5 μg/kg of tissue. The values in μg
per kg were: muscle, 2.7, 3.0; liver, 5.1 ± 1.4 (SD); kidney, 4.4 ± 1.0 (SD). Thus there was some evidence of uneven distribution of the residues of parent drug within an organ.

In another study, eight pigs (90-100 kg) were injected intramuscularly with carazolol at a dose of 10 µg per kg of body weight and slaughtered 1.75 hours after injection. The carazolol content in the kidneys was measured by HPLC; after correcting for recovery, a mean value ± SD of 19 ± 4 µg per kg was found.

In the radiometric studies, the highest concentrations of residues in tissues were in the liver, kidney, and lungs, with generally much lower levels in the muscle, fat, and brain. In the first study (see Table 1), and excluding the injection site, the values were highest at 2 hours (the first time point) after dosing, except in the fat, where the maximum value was at 8 hours. Residues were present in all the tissues at 16 hours after dosing. No information is available on the identity of the residues but, in view of the extensive metabolism of β-adrenoceptor blockers, it might be assumed that a high proportion of the residues in liver and kidney are metabolites and not parent drug. There is some indication, based on data for one pig, that the residues in muscle may be parent drug, while in liver and kidney parent drug accounts respectively for about 15% and 20% of the total residues.

Cattle. Dosing of cattle by intramuscular (i.m.) and intravenous (i.v.) injection gave a similar distribution of residues of parent drug (Table 2); the pattern was also similar to that seen in pigs (see Table 1).

A total of 12 cows were collected in a slaughterhouse; six were injected intramuscularly with 10 mg of carazolol and the remainder in an abdominal vein with 5 mg of carazolol. The cows were slaughtered at various time intervals after injection, and samples of liver, kidney, muscle, and fat collected for determination of carazolol by HPLC with fluorescence detection and a limit of detection of 0.5 µg/kg of tissue. The

<table>
<thead>
<tr>
<th>Withdrawal time (hours)</th>
<th>Weight of animal (kg)</th>
<th>Residue (µg/kg of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.m.</td>
<td>i.v.</td>
</tr>
<tr>
<td>2.75</td>
<td>255</td>
<td>215</td>
</tr>
<tr>
<td>4.5</td>
<td>203</td>
<td>199</td>
</tr>
<tr>
<td>7.75</td>
<td>241</td>
<td>243</td>
</tr>
<tr>
<td>14.0</td>
<td>227</td>
<td>256</td>
</tr>
<tr>
<td>18.0</td>
<td>221</td>
<td>272</td>
</tr>
<tr>
<td>23.5</td>
<td>232</td>
<td>250</td>
</tr>
</tbody>
</table>
weights of the cows, their treatment and the results in terms of residues of carazolol are given in Table 2.

The residues found after intramuscular and intravenous administration did not essentially differ at the two doses used. The results were combined and the mean elimination half-lives of carazolol calculated. The values were: muscle, 8.9 hours; liver, 7.8 hours; and kidney, 4.9 hours.

Analytical control methods
Several chromatographic methods are used by regulatory authorities for detecting and determining residues of carazolol in porcine and bovine tissues (usually kidney) and fluids. Carazolol is extracted from the tissues or fluids, purified by either thin-layer chromatography (TLC) or HPLC and measured by ultraviolet or fluorescence spectroscopy. The lower limits of detection are in the low μg per kg or litre range. When the tests were carried out on tissues collected from pigs treated with carazolol approximately 2 hours before slaughter, residues of carazolol were detected in liver and kidneys but were usually undetectable in muscle or fat.

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

- A temporary ADI of 0-0.1 μg per kg of body weight was established. This would result in a maximum ADI of 6 μg for a 60-kg human.
- The metabolites/residues other than carazolol do not contribute significantly to the biological β-adrenoceptor-blocking activity of the residues.
- All the residues in muscle and fat were considered to be parent drug.
- If the limited information obtained from the study with one pig is taken into account, a major fraction of the total residues in liver and kidney was not parent drug.

The Committee recommended temporary MRLs for carazolol of 5 μg/kg for muscle and fat and 30 μg/kg for liver and kidney in both cattle and pigs. This would result in a maximum daily intake of 6.25 μg if the daily intake values adopted at the thirty-fourth meeting of the Committee are used (Annex 1, reference 85). This value is slightly greater than the temporary ADI but, because the consumption data are at the upper limit of the range for individual intake of animal products, the Committee concluded that the recommended temporary MRLs do not present a risk for the consumer.

The Committee requires the results of the following studies for evaluation in 1994:

1. A study to define a pharmacological no-effect level in humans.
2. Radiometric studies on the concentrations of carazolol and its metabolites as proportions of the total residue in pigs and cattle over a 24-hour period.
3. Nonradiometric studies on carazolol residues in pigs, using suitable analytical methods, over a 24-hour period.

3.2 Anthelmintic agents

The Committee considered the three anthelmintic agents fenbendazole, oxfendazole, and febantel. Of these, the first two are benzimidazoles that are metabolically interconvertible in vivo. Febantel is a prodrug that can be converted in vivo by cyclization to fenbendazole or following oxidation at the sulfur atom and subsequent cyclization to oxfendazole (see Fig. 1).

3.2.1 Febantel

Febantel had not previously been reviewed by the Committee.

Toxicological data

The toxicological data considered by the Committee included the results of studies on metabolism, of acute and short-term studies, and of studies of carcinogenicity, genotoxicity, reproduction, and development.

Figure 1
Metabolism of febantel, fenbendazole, and oxfendazole

![Diagram of metabolites of febantel, fenbendazole, and oxfendazole]
The main route of metabolism in rats, sheep, and cattle appears to be cyclization to yield fenbendazole. Oxidation at the sulfur atom can also occur to yield the sulfoxide, which then undergoes cyclization to give oxfendazole. Both fenbendazole and oxfendazole can then undergo further metabolism.

In a study in rats in which febantel was given by gavage at doses of up to 125 mg per kg of body weight per day for 7 days, the only drug-related effect was fatty infiltration of the liver. The NOEL was 50 mg per kg of body weight per day. Dogs were given febantel daily in gelatin capsules for 13 weeks at doses of up to 180 mg per kg of body weight per day. Dogs given 60 and 180 mg per kg of body weight per day showed testicular hypoplasia and reductions in haematocrit, haemoglobin, and erythrocyte counts. Leukopenia was noted in all treated groups, although the effect was not dose-related. Agranulocytosis was noted in the high-dose dogs, and one high-dose animal out of six also had splenic atrophy. In an extension of the study using doses of up to 10 mg per kg of body weight per day, no haematological, splenic, or testicular effects were seen. The NOEL was 10 mg per kg of body weight per day. In a 52-week study in dogs given febantel at levels of up to 1 g per kg in the diet (equivalent to 25 mg per kg of body weight per day), reductions in haematocrit, haemoglobin, and erythrocyte counts occurred at the highest dietary level. Testicular and lymphofollicular (including splenic) atrophy also occurred at this dietary level. The NOEL was 200 mg per kg in the diet, equivalent to 5 mg per kg of body weight per day.

A carcinogenicity study was conducted in mice, which received febantel in the diet at levels of up to 170 mg per kg of body weight per day for males and 250 mg per kg of body weight per day for females for up to 21 months. There were no changes in tumour incidence as compared with control values.

In a combined long-term toxicity and carcinogenicity study, rats were given febantel in the diet at levels of up to 40 mg per kg of body weight per day during an 11-week period prior to mating, during a 20-day mating period, and during lactation. A total of 60 male and 60 female pups from each dose group was then given the same dietary level as the parents for up to 30 months. Females given the highest dietary level showed reductions in mean corpuscular haemoglobin and increases in absolute and relative liver weights. These animals had fatty vacuolation of the liver at histopathological examination both at week 65 after weaning and at the end of the study. There was no increase in tumour incidence, but the Committee recognized that the doses used may not have been sufficiently high for the carcinogenic potential of this compound to have been adequately explored. The NOEL for non-neoplastic effects was 8 mg per kg of body weight per day.

In a two-generation study in which rats were given febantel in the diet at up to 50 mg per kg of body weight per day, litter size and viability of the young
were reduced at the highest dietary level in both generations. The NOEL for reproductive performance was 10 mg per kg of body weight per day; however, if hepatic hypertrophy and glycogen deposition were taken into account, the overall NOEL was 2 mg per kg of body weight per day.

In a teratogenicity study in rats, severe maternal toxicity and embryotoxicity occurred at the highest dose level. The NOEL was 22 mg per kg of body weight per day. Teratogenicity was not seen in the absence of maternal toxicity and embryotoxicity.

The genotoxic potential of fentanet was investigated in a number of test systems. Negative results were reported in two Ames tests, in a test for DNA repair in bacteria, in an in vivo cytogenetic test in the hamster, and in the mouse micronucleus test. However, a positive result was found in the dominant lethal test in the mouse. Two metabolites of fentanet, fenbendazole and 2-amino-5-phenylsulfanyl-2-benzimidazole (the amino derivative of oxfendazole), also gave negative results in the Ames test and in a test for DNA repair in primary rat hepatocytes, but both gave positive results in the mouse lymphoma tk-locus genotoxicity assay in the presence of metabolic activation, though not in its absence.

No studies of fentanet in humans were available to the Committee.

The Committee noted that no carcinogenic effects were seen in rats and mice; the significance of the positive results found with the compound and its metabolites in a small number of tests for genotoxic potential was therefore unknown. The major effects were those noted in the 13- and 52-week studies in dogs, in the teratogenicity studies in rats, where severe maternal toxicity and embryotoxicity were noted, and in the two-generation study in rats, which was the most sensitive study and gave a NOEL of 2 mg per kg of body weight per day.

The Committee established a temporary ADI of 0-10 μg per kg of body weight for fentanet using a safety factor of 200. The ADI was made temporary because of the inadequate dosing in the rat carcinogenicity study.

Even though a temporary ADI was established, it was not used for recommending MRLs. Before the toxicological issues relating to this compound can be resolved, additional information on its genotoxic and carcinogenic potential will have to be provided (see section 3.2.4).

Residue data
Fentanet is rapidly absorbed from the gut, peak levels in the blood reaching a maximum within a few hours of dosing.

After oral dosing of rats, 17-30% of the dose is excreted in the urine and 70% in the faeces. No data were available on the excretion of fentanet in other species.

The metabolism of fentanet was extensively studied in four species, using the 14C-labelled compound. Fentanet rapidly cyclizes in vivo to yield
fenbendazole, oxfendazole, and oxfendazole sulfone as its main metabolites. There is some evidence of parent drug and its sulfone in liver tissue at very short withdrawal times.

Metabolite profiles were determined in the urine of rats, the urine, faeces, and serum of sheep and pigs, and the liver of rats and cattle. The metabolites excreted by the three species were generally similar but the quantities differed slightly. A summary of the profiles is shown in Table 3.

The metabolites in the liver tissue of rats and cattle are similar at early withdrawal times, but in bovine liver, where residues were measured at a 10-day withdrawal time, a much higher proportion was in the bound form. At a withdrawal time of less than 1 day, the residues could readily be extracted in the free form.

About half the residues in rat liver 1 hour after dosing were fenbendazole and 4-hydroxyfenbendazole, with oxfendazole sulfone, oxfendazole, oxfendazole sulfone amine, and febantel as minor residues.

In bovine liver 18 hours after dosing with febantel, 90% of the residues were readily extractable; the main metabolites (expressed as % total residues in liver) were fenbendazole, 30-41%; oxfendazole, 4-19%; oxfendazole sulfone, 14-15%; and febantel, 3-6%. Traces (1-3%) of the amines of oxfendazole, oxfendazole, and oxfendazole sulfone were also recorded.

Because less than 25% of the residues were readily extracted from bovine liver 10 days after dosing, more vigorous techniques of solvolysis were

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Urine</th>
<th>Faeces</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sheep&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pig&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Febantel</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fenbendazole</td>
<td>0</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Oxfendazole</td>
<td>27-36</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Oxfendazole sulfone</td>
<td>0</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Fenbendazole amine</td>
<td>0</td>
<td>0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Oxfendazole amine</td>
<td>10-13</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Oxfendazole sulfone amine</td>
<td>14-18</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>4-Hydroxyfenbendazole</td>
<td>8-13</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> 0: below limit of quantification.
<sup>b</sup> One hour after dosing with 25 mg/kg of body weight.
<sup>c</sup> Up to 72 hours after dosing with 5 mg/kg of body weight.
<sup>d</sup> Up to 48 hours after dosing with 5 mg/kg of body weight.
<sup>e</sup> At 24 hours after dosing.
Table 4
Total drug-related residues of febantel in animals given [¹⁴C]febantel as a single oral dose*

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep (5 mg/kg of body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.00</td>
<td>0.235</td>
<td>0.035</td>
<td>0.100</td>
</tr>
<tr>
<td>8</td>
<td>2.50</td>
<td>0.075</td>
<td>&lt;0.010</td>
<td>0.015</td>
</tr>
<tr>
<td>15</td>
<td>0.30</td>
<td>0.045</td>
<td>&lt;0.010</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Cattle (7.5 mg/kg of body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>10.7</td>
<td>2.7</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>14</td>
<td>0.6</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>28</td>
<td>0.3</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>42</td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Pigs (5 mg/kg of body weight)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.35</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>NA</td>
</tr>
<tr>
<td>20</td>
<td>0.15</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>NA</td>
</tr>
<tr>
<td>30</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NA</td>
</tr>
</tbody>
</table>

* NA: not available.

used. These yielded residues that were predominantly the oxendazole sulfone amine (12-35% of total residues), together with much smaller amounts of fenbendazole, oxendazole, oxendazole sulfone, and oxendazole amine. The amines may be formed as artefacts during solvolysis.

[¹⁴C]Febantel depletion studies have been carried out in rats, sheep, cattle, and pigs. The liver contained the highest levels of residues, with slightly less in the kidney tissue and much less in muscle and fat. The total residues in the edible tissues of sheep, cattle, and pigs are shown in Table 4.

Residues were also determined after the administration of nonlabelled febantel to sheep, cattle, and pigs by measuring the combined concentrations of fenbendazole, oxendazole, and oxendazole sulfone in the edible tissues (Table 5). The Committee concluded that liver was the most suitable target tissue for the determination of residues.

Analytical methods were reported for measuring concentrations of fenbendazole, oxendazole, and oxendazole sulfone either separately or as the sulfone after oxidation of the sulfide and sulfoxide with permanganate. The oxidation method consists of the following stages: homogenization, extraction, oxidation, solvent partitioning, and quantification of fluorescence. Recoveries ranged from 66% to 90%, with background levels for all tissues of <0.01 mg/kg.

For a discussion of MRLs, see section 3.2.4.
Table 5

**Total residues of fenbendazole, oxfendazole sulfone, and oxfendazole in edible tissues, measured as oxfendazole sulfone equivalents**

<table>
<thead>
<tr>
<th>Species and dose</th>
<th>Withdrawal time (days)</th>
<th>Total residues (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Sheep</td>
<td>7</td>
<td>2.2</td>
</tr>
<tr>
<td>(5 mg per kg of body weight)</td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cattle</td>
<td>7</td>
<td>0.8</td>
</tr>
<tr>
<td>(10 mg per kg of body weight)</td>
<td>14</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.2</td>
</tr>
<tr>
<td>Pigs</td>
<td>12</td>
<td>0.1-1.0</td>
</tr>
<tr>
<td>(5 mg per kg per day for 6 days)</td>
<td>20</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0.05</td>
</tr>
</tbody>
</table>

3.2.2 *Fenbendazole*

Fenbendazole had not previously been reviewed by the Committee.

*Toxicological data*

Comprehensive toxicological data on fenbendazole were provided, including the results of studies on its kinetics, metabolism, short- and long-term toxicity, carcinogenicity, genotoxicity, reproductive toxicity, embryotoxicity, and teratogenicity.

The rate of absorption following oral administration was slow, but more rapid in monogastric animals. The extent of absorption was 25-50% in rats, less than 20% in dogs, 70% in rabbits, 25% in sheep, and more than 33% in pigs. Elimination was greater than 90% within 3 days, with the majority in the faeces. Fenbendazole was metabolized to oxfendazole (the sulfoxide), oxfendazole sulfone, and amine metabolites, which were detectable in plasma. The major urinary metabolite was 4-hydroxyfenbendazole, with traces of oxfendazole and oxfendazole sulfone. The metabolic pathway was similar in rats, rabbits, dogs, sheep, cattle, goats, and chickens.

In a 24-month study in mice in which fenbendazole was given in the diet, there were sporadic body-weight differences between treated and control groups but no meaningful relationship with drug treatment. Survival was reduced in the treated groups, but only at the highest dose of 405 mg per kg of body weight per day could it be attributed to fenbendazole administration. There were no increases in tumour incidence. The NOEL was 135 mg per kg of body weight per day.

Rats born during a multigeneration study received fenbendazole in the diet for 123 weeks at doses of 5, 15, 45, and 135 mg per kg of body weight per
day. Diarrhoea was observed at 45 and 135 mg per kg of body weight per day; weight gain was reduced at these doses and in females at 5 mg per kg of body weight per day. Lymph nodes were affected at all but the low dose of 5 mg per kg of body weight per day, showing enlargement or cyst formation, sinus dilatation, and reactive hyperplasia. The incidence of testicular interstitial-cell adenomas was increased in males at the dose level of 135 mg per kg of body weight per day. The major target organ was the liver, which was affected at and above 15 mg per kg of body weight per day. The following alterations were noted: increased serum alkaline phosphatase activity, hepatocellular hypertrophy and hyperplasia, bile duct proliferation and biliary cysts, and cytoplasmic vacuolation. There was a slight increase in the incidence of hepatocellular carcinomas in females at 135 mg per kg of body weight per day.

The Committee noted that the liver histopathology slides from the chronic toxicity study in rats had been assessed three times and, although there were some differences in criteria and terminology, a consensus had been reached. The incidence of hepatocellular carcinomas was very low, and there was no statistically significant increase as compared with controls. Nevertheless, given the extremely low incidence in concurrent and historical controls, tumours found in females receiving the highest dose of fenbendazole may have been related to treatment. It was noted that the small increase in carcinomas was observed against a background of statistically significant focal hyperplasia. The NOEL in this study was found to be 5 mg per kg of body weight per day, based on pathological changes in the liver and lesions in the lymph nodes.

In a series of studies in dogs, fenbendazole was administered in capsules for periods of 6 days to 6 months. The major toxic effect was lymphoid hyperplasia in the gastric mucosa and mesenteric lymph nodes, resulting in an overall NOEL of 4 mg per kg of body weight per day. This effect was considered by the Committee to be less important than the changes seen in the liver of rats.

A three-generation reproduction study was conducted in rats given fenbendazole in the diet at doses of 5, 15, 45, and 135 mg per kg of body weight per day. Toxic effects in adult animals, including diarrhoea, reduced weight gain, and pathological changes in the liver, were observed at and above 45 mg per kg of body weight per day. At these doses there were also reductions in fertility, survival, and growth of neonates during lactation. The NOEL was 15 mg per kg of body weight per day.

Fenbendazole was tested for embryotoxicity and teratogenicity in rats and rabbits dosed by gavage. Embryotoxicity was not seen in either species, while fetotoxicity in the form of an increased frequency of occurrence of 13th ribs and delayed ossification of cranial bones occurred in rabbits given a dose of 63 mg per kg of body weight per day. The NOELs were 2500 mg per kg of body weight per day in rats and 25 mg per kg of body weight per day in rabbits.
In dogs, pigs, sheep, and cattle, the oral administration of fenbendazole at various times during the gestation period did not result in treatment-related effects in the offspring.

Fenbendazole did not produce mutations in bacteria or chromosomal aberrations in two different in vivo tests. It increased the mitotic index of HeLa cells in vitro, an effect that could be related to the ability of benzimidazoles to interfere with tubulin polymerization and thus inhibit spindle formation.

The most significant toxicological findings with fenbendazole were in the rat liver. Since fenbendazole appears to be nongenotoxic, the Committee considered that a threshold would exist for these effects. Thus a NOEL was based on the absence of histopathological changes in the liver at 5 mg per kg of body weight per day in the long-term toxicity/carcinogenicity study in rats.

A temporary ADI of 0-25 µg per kg of body weight was established based on the NOEL of 5 mg per kg of body weight and the application of a safety factor of 200.

Even though a temporary ADI was established, it was not used for recommending MRLs. Before the toxicological issues relating to this compound can be resolved, additional information will have to be provided to explain the mechanism of the observed increased incidence of tumours in female rats at high doses, including the results of a study of in vivo DNA binding in the rat liver following oral administration of fenbendazole.

Residue data

The Committee considered information on the residues of fenbendazole in cattle, sheep, and pigs. Typical single therapeutic doses for cattle, sheep, and pigs are 10, 5, and 5 mg/kg of body weight, respectively. Considerable variations in blood and tissue levels are found in cattle after oral administration of fenbendazole, depending on whether it is given as a suspension or in the form of pellets.

After oral administration of [14C]fenbendazole to cattle, almost 77% of the applied dose was recovered in faeces, 14% in urine, and 0.3% in the milk. In sheep, the proportion excreted in the faeces and urine after oral dosing averaged 95% and 7%, respectively. In pigs given [14C]fenbendazole orally 50-60% of the radioactivity was recovered in the first 3 days in the faeces and 30-35% in the urine. A far higher proportion of the administered parent fenbendazole (37-52%) was found in faeces than in urine (only 1%).

Fenbendazole is metabolized by oxidation of the sulfide molecule, hydroxylation of the phenyl ring, and degradation of the carbamate to the amine. In all species studied, the main residues found in edible tissues were the parent drug, fenbendazole sulfoxide (oxfendazole), and oxfendazole sulfone.
Cattle. Metabolism studies, in which cattle were treated with $[^{14}C]$fenbendazole at 10 mg per kg of body weight, demonstrated that 80% of the radioactivity of the liver was associated with the parent compound 24 hours after administration, while 7 days after dosage, two major residues were present, namely, fenbendazole and oxfendazole. The highest concentrations were present in the liver, followed by the kidney, with lower concentrations in the fat and muscle. Residue-depletion studies in cattle dosed with unlabelled fenbendazole at 10 mg per kg of body weight showed tissue concentrations of fenbendazole, after 2 days withdrawal, of 8.4 mg/kg in liver, 1.04 mg/kg in kidney, 0.47 mg/kg in muscle, and 0.95 mg/kg in fat; after 7 days withdrawal, only liver had residues (of 0.67 mg/kg) detectable by a fluorimetric method. A further study, in which three cattle were given fenbendazole at 7.5 mg per kg of body weight, showed liver concentrations for fenbendazole, oxfendazole, and oxfendazole sulfone after 7 days withdrawal of 1.29 mg/kg, 1.92 mg/kg, and 0.08 mg/kg, respectively. At that time, most residues were protein-bound.

Milk. $[^{14}C]$Fenbendazole was given orally to six lactating cows at 10 mg per kg of body weight as a 2.5% suspension. The highest levels in milk (1.29 mg/l) were found during the second and third milking; 1 week after administration they had fallen to <0.1 mg/l. The levels of fenbendazole in milk varied widely from one animal to another, but generally the level of oxfendazole exceeded that of fenbendazole; the sulfone concentration was comparable to the lower of those for fenbendazole or oxfendazole. The maximum concentrations of all three combined exceeded 1 mg/l.

Sheep. No radiometric studies were reported in sheep, but results obtained by means of unlabelled residue-depletion studies were similar to those just given for cattle. In the liver, oxfendazole levels were almost double those of fenbendazole and ten times as high as those of oxfendazole sulfone at 7 days withdrawal.

Pigs. Following oral administration of $[^{14}C]$fenbendazole at 3 mg per kg of body weight as a 2.5% suspension, total residues (mg/kg) amounted to 0.63, 0.96, 0.01, and 0.01 in liver, kidney, muscle, and fat, respectively. In a study on pigs treated with unlabelled fenbendazole at 5 mg per kg of body weight a concentration of 0.28 mg/kg of the drug at 7 days withdrawal was found in the liver. Concentrations in other tissues, muscle, kidney, and fat were below the limit of detection of the fluorimetric method employed.

A relay bioavailability study was carried out in rats, which were fed cattle liver containing residues of $[^{14}C]$fenbendazole at concentrations of 2.66 mg/kg and 1.0 mg/kg. The concentrations in all tissues examined, with the exception of the gastrointestinal contents, were below the sensitivity limit of 0.01 mg/kg. The $^{14}$C-labelled substance is not readily bioavailable at low levels.

Analytical methods are available for the determination of fenbendazole in milk. Fluorimetry at a level of 0.25 mg/kg and HPLC at a limit of detection
of 0.05 mg/kg have been used. Similarly, for tissue, fluorimetry (limit of detection 0.1 mg/kg) and validated HPLC methods (limit of detection 0.02 mg/kg) may be used.

For a discussion of MRLs, see section 3.2.4.

3.2.3 Oxfendazole

Oxfendazole had not been previously reviewed by the Committee. The compound is used in cattle, sheep, and horses.

Toxicological data
The results of toxicological studies, including: pharmacokinetic studies on rats, cattle, and sheep; carcinogenicity studies on mice and rats; mutagenicity, embryotoxicity, and teratogenicity studies on mice, rats, rabbits, sheep, cattle, pigs, and horses; studies on eye and skin irritation in rabbits and guinea-pigs; reproduction studies on rats; and short-term and long-term studies on mice, rats, and dogs, were considered by the Committee.

Pharmacokinetic data have demonstrated very good absorption of orally administered oxfendazole (100% in rats, 77% in cattle, and 85% in sheep). The results showed that, after administration of the drug, the plasma metabolite pool was composed of oxfendazole, oxfendazole sulfone, and fenbendazole. There was very little difference between the various animal species, apart from quantitative differences in the plasma metabolite pool. Oxfendazole is excreted in urine and faeces. The compound and its metabolites can undergo enterohepatic circulation.

The genotoxic potential of the compound was tested in an Ames test using only four strains of Salmonella typhimurium; this gave negative results both with and without metabolic activation.

Carcinogenicity studies were performed in mice and rats. Mice received a diet containing oxfendazole in doses up to 1000 mg/kg over a period of 78 weeks. Except for hepatocytic lipid vacuolation, no effects related to oxfendazole treatment were observed. The NOEL for this study was 300 mg/kg in the diet, equivalent to 45 mg per kg of body weight per day.

In the rat carcinogenicity study, animals received oxfendazole in the diet at concentrations of up to 100 mg/kg for 2 years. The only dose-related effect was seen in animals receiving 30 and 100 mg/kg, which showed hepatocellular lipid vacuolation; this was the earliest sign of compound-related effects seen in the liver in this study. The NOEL was 10 mg/kg in the diet, equal to 0.7 mg per kg of body weight per day in males and 0.9 mg per kg of body weight per day in females.

The Committee noted that two different strains of mice were used in the range-finding and carcinogenicity studies. It was concluded, however, that this difference was not of great significance since Swiss mice were used in both studies. There was no evidence of any carcinogenic effect in rats.
However, the Committee was of the opinion, based on the 90-day range-finding study in rats, that higher doses could have been used.

In a two-generation reproduction study in rats in which oxfendazole was administered in the diet, no effect was observed on mating behaviour or fertility, maternal behaviour, length of gestation, live litter size, gestation index (number of fetuses per female), or survival of offspring. However, the proportion of pregnancies in the second-generation females receiving 100 mg/kg was significantly lower than in controls. The NOEL in this study was 10 mg/kg in the diet, equal to 0.9 mg per kg of body weight per day.

Oxfendazole did not produce irritation when tested in the rabbit eye or skin, or sensitization effects in guinea-pig skin.

The Committee considered data from embryotoxicity and teratogenicity studies of oxfendazole conducted on mice, rats, rabbits, sheep, cattle, pigs, and horses.

A dose of 360 mg per kg of body weight per day was fetotoxic in mice. The NOEL in mice was 108 mg per kg of body weight per day.

Results were reported from three teratogenicity studies in sheep given doses of oxfendazole ranging from 7.5 to 22.5 mg/kg of body weight on days 12, 14, 17, 20, or 23 of gestation. The sheep fetus was most susceptible to the induction of teratogenic effects on day 17, when the NOELs ranged from 7.5 to 15 mg per kg of body weight.

A teratogenicity study was performed in New Zealand rabbits at doses up to 0.625 mg per kg of body weight per day administered on days 6-18 of gestation. No signs of maternal toxicity or effects on reproduction indices were reported. A number of minor soft-tissue and skeletal changes were seen in fetuses from treated animals, but these were thought not to be treatment-related, and the study gave a NOEL of 0.625 mg per kg of body weight per day. While the Committee recognized that rabbits may be relatively sensitive to benzimidazole-related toxic effects, it believed that the doses used in this study were not high enough to enable the teratogenic potential of oxfendazole in the rabbit to be adequately explored.

A temporary ADI of 0-4 μg per kg of body weight was established for oxfendazole, based on a NOEL of 0.7 mg per kg of body weight per day from the carcinogenicity study in rats and the use of a safety factor of 200.

The Committee noted that, if the NOEL for teratogenicity in sheep had been used as the basis for establishing the temporary ADI together with a safety factor of 2000, the value obtained would have been of the same order as that derived from the carcinogenicity study in rats.

A temporary ADI was established because of concern that sufficiently high doses had not been used in the carcinogenicity study in rats and the teratogenicity study in rabbits and the lack of genotoxicity data.
Residue data
The Committee considered data on the metabolism of oxfendazole and the depletion of residues of oxfendazole from edible tissue of cattle, sheep, and horses.

The absorption, distribution, metabolism, excretion, and tissue residues of oxfendazole in cattle, sheep, and horses have been studied using [14C]oxfendazole and are qualitatively similar in all three species. Oxfendazole was excreted primarily in the faeces following oral administration. Orally administered oxfendazole was well absorbed by cattle and sheep. The extent of oral absorption by horses has not been studied but was expected to be high.

For all species, liver was the tissue with the highest concentration of drug-related residue and slowest rate of residue depletion. The extractable portion of the residue present in liver consisted of oxfendazole, oxfendazole sulfone, and fenbendazole. A large portion of the residue present in liver was not extractable and this proportion increased with time after dosing. Studies demonstrated that the bound residue has low bioavailability.

Cattle. A total of 24 calves, approximately 6 months of age and 160-210 kg in weight, were dosed orally once with a suspension of [13C]oxfendazole at the rate of 5 mg per kg of body weight. The average total residue levels are shown in Table 6.

The liver tissues were assayed by HPLC for combined oxfendazole, fenbendazole, and oxfendazole sulfone. The average residue levels were 1.116, 0.026, 0.005, and 0.003 mg/kg at 7, 14, 21, and 28 days after dosing. The HPLC assay has a claimed level of reliable measurement of 0.002 mg/kg.

Examination of the 14C-labelled residue present in the livers of treated animals showed that, 3 days after administration of oxfendazole, 76% of the total residue was extractable and that, of the extractable residue, 68% was oxfendazole, 12% fenbendazole, and 15% the sulfone. Of the residue

<table>
<thead>
<tr>
<th>Time after dosing (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.041</td>
<td>5.438</td>
<td>0.954</td>
<td>0.040</td>
</tr>
<tr>
<td>14</td>
<td>0.010</td>
<td>2.372</td>
<td>0.249</td>
<td>0.009</td>
</tr>
<tr>
<td>21</td>
<td>0.005</td>
<td>1.245</td>
<td>0.097</td>
<td>0.011</td>
</tr>
<tr>
<td>28</td>
<td>0.004</td>
<td>0.723</td>
<td>0.056</td>
<td>0.010</td>
</tr>
</tbody>
</table>

* Each figure is the mean value for six calves.
present 7 days after dosing, 23% was extractable and, of the extractable residue, 55% was oxfendazole, 6% fenbendazole, and 8% sulfone.

Six cattle (3 males and 3 females) were given an intraruminal dose of oxfendazole at 4.5 mg/kg of body weight. The concentrations of oxfendazole, fenbendazole, and oxfendazole sulfone were determined separately in liver by an HPLC method that has a quantification limit of 0.005 mg/kg for each analyte. At 7 days after dosing, the combined concentration of these three metabolites was 0.125 mg/kg.

A study was conducted on the bound residue fraction of the oxfendazole total residue in cattle liver, using the Gallo-Torres bioavailability model. The "dosed" liver tissue was obtained from a beef steer 209 kg in weight. The animal was given a single dose of [\(^{14}\text{C}\)]oxfendazole at 4.5 mg/kg of body weight by injection and was killed 7 days later. The level of total radioactivity present in the liver was 2.94 mg/kg. The extractable residue was extracted with three portions of ethyl acetate. The total extractable residue was 16.7% of the total residue.

The bioavailability in the rat of the nonextractable residue (83% of the total residue) was 9%. Under the same experimental conditions, the bioavailability of oxfendazole present in liver tissue spiked with [\(^{14}\text{C}\)]oxfendazole was 70%. These values yield a bioavailability of 13% for nonextractable residues relative to nonextractable parent drug.

A total of 36 cattle were dosed intraruminally with oxfendazole at 4.5 mg/kg of body weight. The concentrations of oxfendazole, fenbendazole, and the sulfone in the livers were separately determined by an HPLC assay with a quantification limit of 0.005 mg/kg for each analyte. The results of this study are summarized in Table 7.

Three lactating cows were given a dose of 2.5 mg/kg of body weight of [\(^{14}\text{C}\)]oxfendazole by gavage. The concentrations of drug equivalents in the milk were determined over a period of 13 days after dosing. The concentration of residue was highest 1 day after dosing (0.49 mg/kg) and was below 0.005 mg/kg 8 days after dosing. The extractable fraction constituted about 50% of the total radioactivity over a 72-hour period. In the 0-24 hour pool, the extractable fraction consisted of 70% oxfendazole and 30% reduced or oxidized metabolites. The principal metabolite in the 48-72 hour pool was the oxidized species oxfendazole sulfone.

<p>| Table 7 |
|---|---|---|---|
| Concentrations (mg/kg) of oxfendazole, fenbendazole, and oxfendazole sulfone in liver of cattle given oxfendazole at 4.5 mg/kg of body weight |</p>
<table>
<thead>
<tr>
<th>Time after dosing (days)</th>
<th>Oxfendazole</th>
<th>Fenbendazole</th>
<th>Oxfendazole sulfone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.042</td>
<td>0.077</td>
<td>0.008</td>
<td>0.125</td>
</tr>
<tr>
<td>14</td>
<td>&lt;0.005</td>
<td>0.006</td>
<td>0.005</td>
<td>0.016</td>
</tr>
</tbody>
</table>
Table 8
Total radioactivity (mg oxfendazole equivalents per kg) in tissues of sheep treated with a single oral dose of \[^{14}C\]oxfendazole at 6 mg/kg of body weight

<table>
<thead>
<tr>
<th>Time after dosing (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.02</td>
<td>7.34</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>14</td>
<td>0.01</td>
<td>1.31</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>21</td>
<td>0.01</td>
<td>0.27</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Study 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.030</td>
<td>2.479</td>
<td>1.095</td>
<td>0.195</td>
</tr>
<tr>
<td>13</td>
<td>0.080</td>
<td>2.580</td>
<td>0.011</td>
<td>0.070</td>
</tr>
<tr>
<td>23</td>
<td>0.006</td>
<td>0.218</td>
<td>0.044</td>
<td>0.041</td>
</tr>
<tr>
<td>30</td>
<td>0.002</td>
<td>0.223</td>
<td>0.023</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Six lactating cows were given oxfendazole orally at a dose of 5 mg/kg of body weight. The concentration of oxfendazole in the milk was determined by an HPLC method with a sensitivity of 0.005 mg/kg. The concentration of residues of oxfendazole was highest 1 day after dosing (0.426 mg/kg) and below the quantification limit 84 hours after dosing.

Sheep. Two studies were conducted to determine the total drug-related residue present in tissue following oral administration of \[^{14}C\]oxfendazole to sheep at 6 mg/kg of body weight. The average total residue levels are shown in Table 8.

The residues present in the livers of one group of treated animals were examined and the percentages of extractable residue and of oxfendazole in this extractable residue determined (Table 9). In a bioavailability study of the bound residue in sheep liver, only 8% or less of the bound \[^{14}C\]oxfendazole was absorbed when administered to rats.

A total of 20 sheep were given a single oral dose of oxfendazole at 6 mg/kg of body weight. The concentration of oxfendazole in the liver was determined by an HPLC method with a sensitivity of 0.05 mg/kg. The

Table 9
Extractable residues in liver of sheep given a single oral dose of \[^{14}C\]oxfendazole at 6 mg/kg of body weight

<table>
<thead>
<tr>
<th>Time after dosing (days)</th>
<th>% extractable residues</th>
<th>% oxfendazole in extractable residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>61</td>
<td>82</td>
</tr>
<tr>
<td>14</td>
<td>41</td>
<td>57</td>
</tr>
<tr>
<td>21</td>
<td>7</td>
<td>70</td>
</tr>
</tbody>
</table>
Table 10

Total radioactivity (mg oxendazole equivalents per kg) in tissues of horses treated with a single oral dose of [14C]oxendazole at 10 mg/kg of body weight

<table>
<thead>
<tr>
<th>Time after dosing (days)</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.097</td>
<td>4.461</td>
<td>0.104</td>
<td>0.048</td>
</tr>
<tr>
<td>20</td>
<td>0.002</td>
<td>0.843</td>
<td>0.012</td>
<td>0.009</td>
</tr>
<tr>
<td>30</td>
<td>0.001</td>
<td>0.464</td>
<td>0.014</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Mean levels of oxendazole at 3, 7, 14, 21, and 30 days were 7.86, 3.15, 0.26, 0.08 and ≤0.05 mg/kg, respectively.

Horses. The total drug-related residue present in the edible tissue of horses after oral administration of [14C]oxendazole at a dose of 10 mg/kg of body weight was determined. The average total residue levels are shown in Table 10.

Methods of analysis of residues in tissues

As already mentioned, several HPLC methods are available for measuring oxendazole residues in tissues and milk.

The oxendazole residue in calf liver can be measured by an HPLC method combined with fluorescence detection. The residue measured by this method is a combination of oxendazole, fenbendazole, and oxendazole sulfone, as oxendazole and fenbendazole are oxidized to oxendazole sulfone before analysis. This HPLC assay has a claimed level of reliable measurement of 0.002 mg/kg.

Oxendazole, fenbendazole, and oxendazole sulfone in liver tissue of cattle can be simultaneously quantified by HPLC. This method is stated to have a sensitivity of 5 or 10 μg/kg for each compound.

An HPLC method with ultraviolet detection measures oxendazole concentrations in milk down to levels as low as 0.005 mg/kg. Another HPLC method using UV detection measures oxendazole concentrations in sheep liver, kidney, muscle, and fat down to levels as low as 0.05 mg/kg.

For a discussion of MRLs, see section 3.2.4.

3.2.4 Summary

Toxicological data

The benzimidazoles produce a variety of toxic effects in experimental biological systems, some of which may be due to the well-established binding affinity of 2-substituted benzimidazoles for tubulin, an important component of the cytoskeleton, including the spindle apparatus of dividing cells. Toxicity is frequently observed in haematopoietic tissues, liver, and testis, and there is evidence of embryotoxicity, carcinogenicity, and
genotoxicity for certain benzimidazoles other than those considered by the Committee.

Leukopenia and anaemia accompanying hypocellularity of bone marrow, spleen and thymus have been described in a variety of species given febantel, fenbendazole, or oxfendazole; however, in the case of fenbendazole the effect was limited to leukopenia in pigs. Testicular hypoplasia was observed only with febantel in dogs and with oxfendazole in mice and rats. No such testicular effects were reported for fenbendazole. Embryotoxicity and/or fetotoxicity was observed with all three compounds. Evidence of teratogenicity was found only after the administration of oxfendazole to sheep, although the possibility of a similar effect in rabbits could not be excluded because of the very low doses of oxfendazole used.

Hepatotoxicity was a finding common to the three compounds in several species. A small increase in hepatocellular carcinomas was observed only in female rats treated orally with a high dose of fenbendazole. The absence of similar evidence in experiments with febantel and oxfendazole could be related to the lower administered dose levels of these compounds. It is well known that even a two-fold difference in the dose level is important in the induction of significant responses in rodent carcinogenicity tests.

In tests for genotoxicity, none of these compounds, or 2-amino-5-phenylsulfanyl-2-benzimidazole (a metabolite of oxfendazole) was active in the Ames test or, where tested, in the primary rat hepatocyte DNA repair assay, in in vivo assays for chromosomal aberrations, and the micronucleus test. Significant responses were obtained in the dominant lethal test in male mice with febantel and in the mouse lymphoma tk-locus genotoxicity assay with fenbendazole and its 2-amino metabolite. Fenbendazole was also shown to inhibit mitosis in HeLa cells, a finding that may be important in the interpretation of the significant responses in the genotoxicity tests. Given the generally nongenotoxic properties of these compounds, any hepatocarcinogenic responses are likely to show dose-threshold effects.

Metabolism of febantel, fenbendazole, and oxfendazole results in the presence of a combination of fenbendazole, oxfendazole and their metabolites (Fig. 1, page 13). However, insufficient data were available on the kinetics of the pathways involved and on the degree of absorption from the gastrointestinal tract to allow the Committee to make direct comparisons of the toxicity of each of the compounds on a molar basis after oral administration. The limited quantitative data available on the absorption of these three compounds in the oral carcinogenicity studies in rats suggested that both febantel and oxfendazole were tested at relatively low doses as compared with fenbendazole.

Although no information was presented to identify the causal agent(s) responsible for the range of effects seen with the three compounds, oxfendazole appears to be the most toxic. The Committee therefore
considered that an ADI based on the NOEL for oxendazole would provide the most appropriate basis for proposing MRLs that would fully protect consumers of food containing residues resulting from the administration of any of the three compounds.

Residue data
The Committee considered that a common MRL would be desirable for residues of each of the three drugs for the following reasons:

- The metabolites present as the major residues in edible tissues, namely oxendazole, fenbendazole and oxendazole sulfone, are similar for each drug.
- Residues of febantel are not present after a few days' withdrawal time.
- It is not possible from the analysis of the residues in edible tissues to determine which of the three drugs was administered to the animal.
- The bioavailability of the bound residues, as reported for fenbendazole and oxendazole, is very low so that these residues make an insignificant contribution to the residues of toxicological concern (see section 2.3 of the report of the thirty-sixth meeting of the Committee (Annex 1, reference 9f)).

The Committee recommended that MRLs be determined with the marker residue as the sum of the residues of the principal metabolites — oxendazole, fenbendazole, and oxendazole sulfone — since:

- A suitable method of analysis for the sum of the three metabolites is available for tissues; oxendazole and fenbendazole are readily oxidized to oxendazole sulfone, and the sum of the metabolites is measured as oxendazole sulfone.
- Data on the individual concentrations of oxendazole and fenbendazole residues in edible tissues were very limited.
- Oxendazole and fenbendazole are the substances of toxicological concern. Oxendazole sulfone is not toxic and as it is usually a minor metabolite its inclusion in the MRL makes only a small contribution to the marker residue.

The Committee noted that oxendazole was not indicated for use in pigs and febantel was not indicated for use in lactating cows.

In recommending group MRLs, the Committee took into consideration the temporary ADI for the most toxic compound, oxendazole, of 0.4 µg per kg of body weight, equivalent to a maximum ADI of 240 µg for a 60-kg human.

The recommended temporary MRLs, expressed as the sum of the three metabolites (oxendazole, fenbendazole, and oxendazole sulfone) calculated as oxendazole sulfone equivalents, for cattle, sheep, and pigs, are:

- Muscle, fat, and kidney – 100 µg/kg
- Liver – 500 µg/kg
- Milk (cow) – 100 µg/litre.
These MRLs would result in a maximum daily intake of 240 µg, if the intake values adopted at the thirty-fourth meeting of the Committee are used (Annex 1, reference 85).

Results of the following studies are required for evaluation in 1995:

1. *In vivo* assays for binding to DNA in liver following the oral administration of febantel, fenbendazole, and oxfendazole to rats.
2. Studies designed to explain the activity of fenbendazole and, in particular, 2-amino-5-phenylsulfinyl-2-benzimidazole in the mouse lymphoma *tk*-locus genotoxicity assay.
3. A mouse lymphoma *tk*-locus genotoxicity assay of oxfendazole.
4. Studies that suggest how fenbendazole might induce tumours in female rats at high doses through a nongenotoxic mechanism.
5. Dominant lethal and micronucleus tests on oxfendazole.
6. A teratogenicity study in rabbits using oxfendazole at sufficiently high doses to explore adequately its teratogenic potential in this species.
7. Studies on the total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone), measured as oxfendazole sulfone, in the edible tissues of cattle and sheep and in the milk of cattle over a 28-day withdrawal period after treatment of animals with fenbendazole or oxfendazole. In particular, information is requested on the use of the pelleted form of fenbendazole in cattle and sheep.
8. Studies on the total residues of the above three metabolites, measured as oxfendazole sulfone, in the edible tissues of pigs given fenbendazole and observed over a 7-14-day withdrawal period.
9. Information on the bioavailability of bound residues in liver after administration of febantel to one of the following species: cattle, pigs, or sheep.
10. Development of a suitable method for the determination of total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone, measured as oxfendazole sulfone) in milk.

Quantitative and temporal differences in the metabolism of febantel and oxfendazole in comparison with fenbendazole could mean that these compounds do not act similarly, but the Committee had no evidence on this subject. If it is believed, for metabolic reasons, that requirements 1, 3 and 5 above are not appropriate for febantel and oxfendazole, data may be submitted instead from adequate rat carcinogenicity studies on these compounds.
3.3 Antimicrobial agents

3.3.1 Spiramycin

Spiramycin had previously been evaluated at the twelfth meeting of the Committee (Annex 1, reference 17).

Spiramycin is a macrolide antibiotic used for the treatment and control of a number of bacterial and mycoplasmal infections in animals. It is available as spiramycin embonate for use in animal feed, and as the adipate, a more soluble form, for administration by other routes.

Toxicological and microbiological data

The Committee considered animal pharmacokinetic data, the results of short-term studies in rats, dogs, and monkeys, a carcinogenicity study in rats, teratogenicity studies in mice, rats, and rabbits, and genotoxicity data. It also considered information on pharmacokinetics, adverse reactions, and microbiological effects in humans, and minimum inhibitory concentration (MIC)\(^1\) data.

Limited data suggested that orally administered spiramycin was well absorbed in rats; the same was true in humans. Studies in both animals and humans suggested extensive tissue distribution. In pigs, the highest levels of spiramycin were found in the liver and kidney after dietary administration.

In a short-term dietary study in which rats were given the equivalent of up to 3900 mg per kg of body weight per day for 13 weeks, the only major effects noted were a reduction in neutrophil counts in some mid- and high-dose animals, and dilatation of the caecum; the latter was attributed to antibiotic effects on the rodent gut flora. The NOEL was equivalent to 140 mg per kg of body weight per day. In another dietary study in the rat, animals were given up to the equivalent of 720 mg per kg of body weight per day for 1 year. The only notable effects were reductions in the body weights of females receiving the higher doses, and increases in relative liver, kidney, and adrenal weights at high dose levels in animals of both sexes. Hepatic glycogen depletion occurred at all dose levels but not in controls. However, the significance of this was unknown.

Oral doses of 200 and 500 mg per kg of body weight per day of spiramycin given to dogs for 28 days produced no adverse effects. However, in a second study, when mongrel dogs were given 500 mg per kg of body weight per day for up to 56 days, reductions in spermatogenesis and testicular atrophy occurred. Kidney damage was also seen. All but two of the dogs (out of a total of 20) died before the end of this latter study. A NOEL could not be established, as only a single dose level was used. When beagles were

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\(^1\) The minimum inhibitory concentration is defined as the minimum concentration of an antimicrobial drug giving complete inhibition of growth of a particular microorganism, as judged by the naked eye after a given period of incubation (WHO Technical Report Series, No. 610, 1977).
given dietary spiramycin at up to the equivalent of 150 mg per kg of body weight per day for 2 years, testicular damage was not seen, although degenerative changes occurred in other organs. The NOEL in this study was 75 mg per kg of body weight per day. No adequate reproduction studies were available to the Committee.

In teratogenicity studies in mice, oral doses of spiramycin of up to 400 mg per kg of body weight per day given over days 5-15 of gestation had no effects on the outcome of pregnancy. Intravenous doses of up to 84 mg per kg of body weight per day given on days 6-15 of gestation to rats and days 6-19 to rabbits had no effect on development, but oral doses of 200 and 400 mg per kg of body weight per day in rabbits produced caecal enlargement in mothers and significant embryotoxicity. It is unlikely that these findings in rabbits are of significance for human hazard assessment, because this species is known to be particularly susceptible to the effects of antibiotics on the gut microflora. The embryotoxicity was probably related to maternal toxicity as neither was evident at 100 mg per kg of body weight per day.

The genotoxic potential of spiramycin was investigated in a range of studies. Negative results were obtained with spiramycin adipate and embonate in a forward-mutation test in mammalian cells in vitro, in an in vitro cytogenetic assay, and in the mouse micronucleus test.

There was no evidence of carcinogenicity in the rat when spiramycin was tested in a dietary study at levels equivalent to up to 300 mg per kg of body weight per day over a 2-year period.

Adverse reactions in humans following spiramycin treatment are uncommon but, when encountered, the most frequently reported are mild gastrointestinal disturbances.

In assessing the microbial effects of spiramycin, the Committee considered the results of a study in human volunteers. Six subjects were given 1 g of spiramycin twice a day for 5 days and the effects on microorganisms of the oral cavity investigated. There was no evidence of increased colonization by the microorganisms investigated. Similarly, there were no major changes in the populations of the faecal microorganisms studied. However, MIC values for faecal anaerobes and enterococci increased during treatment, and a NOEL could therefore not be established.

In view of the results of the toxicological studies of spiramycin and those of the human volunteer study on the effects of the drug on the gut flora, the Committee concluded that, of the data available, the results of the in vitro MIC investigations were the most appropriate for use in safety assessment.

Spiramycin is known to be inactive against bacteria of the family Enterobacteriaceae. MICs were available for eight strains representative of four species of the anaerobic dominant flora of the large intestine (Bacteroides, Eubacterium, Clostridium, and Peptostreptococcus species).
Data from recent work indicated that the MICs varied from 0.25 to 2 μg/ml in pure culture at 10^6 bacteria/ml. With increasing density of the bacteria, however, the MIC values also increased significantly. At 10^9 bacteria/ml, the observed MICs for the same strains varied from 2 to >128 μg/ml and were between 8 and 128 times as high as at 10^6 bacteria/ml.

In mixed culture, the MIC of spiramycin for the mixed population was 16 μg/ml at 10^6 bacteria/ml and greater than 128 μg/ml at 10^9 bacteria/ml.

In order to estimate the approximate concentration without antimicrobial effect on the intestinal flora, the following calculations were made (see Annex 5). The modal value of the above in vitro determinations was used as the initial value in subsequent calculations. This modal MIC^1 was reported as 0.5 μg/ml at 10^6 bacteria/ml. In order to cover adequately the range of MICs of sensitive bacteria, this value was divided by a factor of 10.

In the light of data on the influence of bacterial density on MICs, the conditions of co-culture and anaerobiosis, and the unfavourable pH in significant parts of the intestine for the activity of spiramycin, the resulting value was multiplied by 20. This rather conservative factor seemed appropriate in view of the uncertainties involved in the extrapolation of data on the inhibition of bacterial growth from standardized in vitro conditions to the conditions of growth in the gut. As a result of such extrapolation, a concentration without effect on the human intestinal flora of 0.5×20/10 = 1 μg/ml (equivalent to 1μg/g) was estimated.

In human volunteers who had received two oral doses of 1 g of spiramycin daily on five consecutive days, a concentration of 689 ± 48 μg (SD) of spiramycin in faeces was found on the fifth day of treatment. On the basis of a daily faecal bolus of about 150 g, it was possible to estimate the fraction of spiramycin that was bioavailable in active form to the bacteria of the intestine after oral administration. In subsequent calculations, this fraction was assumed to be 5% of the orally ingested dose. In view of the conservative margin of safety already provided by the estimated no-effect concentration, an additional safety factor of 10 was used to cover fully the variability between people of all extrapolated parameters. A temporary ADI was therefore calculated as follows:

\[
\text{Upper limit of temporary ADI (μg/kg of body weight)} = \frac{\text{Concentration without effect on human gut flora (μg/ml)}}{\text{Daily faecal bolus (g)}} \times \frac{\text{Fraction of oral dose bioavailable}}{0.05 \times 10 \times 60}
\]

\[
= \frac{1 \times 150}{0.05 \times 10 \times 60} = 5 \text{ μg per kg of body weight}
\]

^1 For the purpose of this evaluation, the modal MIC means the most frequently observed MIC in a frequency distribution of MICs for strains of the relevant species tested.
Table 11
Concentrations of spiramycin in tissues and at injection sites in cattle

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Concentration of spiramycin (mg/kg)</th>
<th>Muscle</th>
<th>Injection site No. 1</th>
<th>Injection site No. 2</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.09</td>
<td>20.91</td>
<td>35.12</td>
<td>0.48</td>
<td>0.47</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>&lt;0.06</td>
<td>10.23</td>
<td>10.30</td>
<td>0.30</td>
<td>0.17</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>&lt;0.03</td>
<td>0.47</td>
<td>0.60</td>
<td>0.14</td>
<td>0.05</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>&lt;0.03</td>
<td>0.11</td>
<td>0.31</td>
<td>&lt;0.12</td>
<td>&lt;0.03</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>ND</td>
<td>0.13</td>
<td>0.16</td>
<td>&lt;0.12</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>ND</td>
<td>0.05</td>
<td>0.04</td>
<td>&lt;0.06</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* ND: not detected (< 0.015 mg/kg); NS: not sampled.

Residue data

No data were available from residue-depletion studies with radiolabelled spiramycin.

Cattle. A total of 18 young cattle weighing 177-350 kg received two injections of spiramycin at 100,000 IU\(^1\) per kg of body weight at 48-hour intervals. Groups of three animals each were slaughtered at 7-day intervals starting at 14 days after final drug administration. Data on the concentration of spiramycin in tissues and at the injection sites are presented in Table 11.

Concentrations of spiramycin were highest in liver and kidney and lowest in muscle and fat. Detectable residues of spiramycin (<0.06 mg/kg) persisted in liver for 49 days after treatment but quantifiable residues (0.14 mg/kg) only until day 28.

A residue-depletion study was carried out in calves given spiramycin in the feed at 25 mg per kg of body weight for 7 days. Residue levels in muscle and fat decreased in about 3 days to below the detection limit (0.1 mg/kg) of the microbiological method. In contrast, residues persisted in liver and kidney at levels ranging from 15.9 mg/kg at day 3 to below the limit of detection at day 24.

Milk samples were collected for 25 milkings following intramuscular administration of 30,000 IU per kg of body weight of spiramycin to six dairy cows. The concentration of spiramycin was well below 1 mg/litre after 8 milkings (4 days), and at 17 milkings (8.5 days) was 0.09 mg/litre.

Pigs. Residue studies of spiramycin following oral administration were performed in 25-30-kg males and females. Three animals were slaughtered at each withdrawal time. Concentrations of spiramycin and its active metabolites in tissues were determined by the agar diffusion method.

---

\(^1\) The WHO Standard Titre is 3200 IU/mg so that 1 IU is approximately equal to 0.3 \(\mu g\) of spiramycin base.
<table>
<thead>
<tr>
<th>Time after treatment (days)</th>
<th>Concentration of spiramycin (mg/kg)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.58</td>
<td>0.23</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>&lt;0.30</td>
<td>&lt;0.15</td>
<td>ND</td>
<td>&lt;0.10</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>&lt;0.30</td>
<td>&lt;0.15</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>&lt;0.30</td>
<td>&lt;0.15</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* ND: not determined.

with *Micrococcus luteus* ATCC 9341 as test organism. The results, which are summarized in Table 12, show that liver retained the highest concentrations of residues. These decreased to below the limit of quantification (<0.30 mg/kg) after 10 days.

A second residue-depletion study was performed with 15 pigs aged 10-12 weeks after intramuscular injection of 25 mg per kg per day for 3 consecutive days. A microbiological assay with *M. luteus* (limit of detection 0.025 mg/kg) was used. The residue levels were considerably higher in liver and kidney than in other tissues but were not detectable at day 14.

*Poultry.* Spiramycin at a dose of 300 mg/kg of feed was administered to broilers for 10 days. Residues were assessed using a microbiological assay with *M. luteus* (limit of detection 0.02 mg/kg). The results are shown in Table 13.

**Methods of analysis**

Methods are available for determining the concentrations of spiramycin and the microbiologically active metabolite neospiramycin in edible tissues of pigs and cattle as well as in milk. Although significant information on neospiramycin was not available, it was claimed that this metabolite, which is detectable by HPLC, has antibiotic activity of a

<table>
<thead>
<tr>
<th>Time after treatment (days)</th>
<th>Concentration of spiramycin (mg/kg)</th>
<th>Muscle</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.08</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.04</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.02</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td></td>
</tr>
</tbody>
</table>
potency similar to that of the parent compound. The quantification limit for spiramycin by HPLC is 0.1 IU/g (0.03 mg/kg) in muscle and kidney, and 0.2 IU/g (0.06 mg/kg) in liver. For the agar diffusion method, which uses *M. luteus* as the test organism, the quantification limit in milk is 0.2 IU/ml (0.06 mg/l) and in liver and muscle 0.1 IU/g (0.03 mg/kg).

**Maximum Residue Limits**

In recommending temporary MRLs, the Committee took into consideration the temporary ADI of 0.5 µg per kg of body weight, equivalent to a maximum ADI of 300 µg for a 60-kg human. The recommended temporary MRLs are:

- Liver – 300 µg/kg in pigs and cattle
- Kidney – 200 µg/kg in pigs and cattle
- Muscle – 50 µg/kg in pigs and cattle
- Milk – 150 µg/l.

No MRL was assigned for fat because appropriate data were not available.

The temporary MRL allocated to milk takes into account its effect on starter cultures used in yoghurt manufacturing.

The temporary MRLs recommended above would result in a daily maximum intake of 280 µg of spiramycin based on a daily food intake of 1.5 litres of milk and 450 g of combined tissue (excluding fat) (Annex 1, reference 85).

Results of the following studies are required for evaluation in 1994:

1. *In vivo* studies on the effects of spiramycin on the intestinal flora.
2. Radiometric studies on the concentrations of spiramycin and its metabolites as proportions of the total residue in edible tissues of cattle, pigs, and poultry.
3. Studies on the pharmacokinetics of spiramycin residues in the fat of cattle and pigs and the edible tissues of poultry.

**3.3.2 Sulfadimidine**

Sulfadimidine was evaluated at the thirty-fourth meeting of the Committee (Annex 1, reference 85), when a temporary ADI of 0.4 µg per kg of body weight was established. At that time, the Committee was aware of additional studies in progress on the effect of sulfadimidine on the thyroid gland in various animal species, and requested that the results of those studies should be submitted by 1991.

At its present meeting, the Committee was informed that, while those studies had been completed, the final reports were not yet available. Because the results of the studies will be available in the near future, the Committee extended the temporary ADI of 0.4 µg per kg of body weight until 1993.
3.3.3 *Tylosin*

Tylosin was previously evaluated at the twelfth meeting of the Committee (Annex 1, reference 17).

Tylosin is a macrolide antibiotic, active against certain Gram-positive and Gram-negative bacteria and Gram-positive mycoplasmas.

**Toxicological data**

The Committee considered toxicological data on tylosin, including the results of studies on biochemical aspects, mutagenicity, and microbiological activity. The Committee noted that most of the toxicity studies had been carried out about 20 years ago, had not been conducted according to current protocols, and were poorly reported.

Following administration of tylosin by various routes, peak serum levels in rats, dogs, pigs, and cattle were observed within 1-2 hours, and then declined rapidly. In pigs, about 22% was bioavailable after oral administration. Excretion of tylosin was rapid and largely in the bile.

After oral administration of radiolabelled tylosin to rats and pigs, 99% of the radioactivity was excreted via the faeces. Major products identified in faeces were tylosin (factor A), macrosin (factor C), relomycin (factor D), and dihydrodesmycosin. In pig liver and kidney, only very small amounts of tylosin and dihydrodesmycosin could be found.

Several short- and long-term studies in rats and dogs were performed. In a 1-year study in rats, tylosin base was administered in the diet at concentrations up to 10 g/kg of feed. A NOEL of 1 g/kg of feed, equivalent to 50 mg per kg of body weight per day, was established, based on haematological and urinary pH changes. In a study in dogs, tylosin base was administered orally at dose levels up to 400 mg per kg of body weight per day for 2 years. At the two highest dose levels, salivation, vomiting, and diarrhoea as well as mild pyelonephritis were observed. The NOEL was 100 mg per kg of body weight per day.

In two replicate, but not independent, carcinogenicity studies in rats, tylosin base was administered in the diet for 2 years at levels up to 10 g/kg of feed. Food consumption and body-weight gain were increased in both males and females in all treated groups. In male rats, a dose-related increase in pituitary adenomas was observed, from 5% in the controls to 25% in the highest-dose group. There was no such increase in female rats. The authors of the report concluded that the increase in pituitary tumours was an indirect result of the ability of tylosin to increase survival and weight gain. However, this hypothesis was neither tested experimentally nor verifiable in detail by the Committee because individual body weights at about 12 months of age were not available.

No effects on reproduction performance were observed in a two-generation study in mice and in one- and three-generation studies in rats.
No malformations were observed in mice or rats, but the Committee noted that these studies were poorly reported.

Tylosin was not mutagenic in an in vitro test for chromosomal aberrations and in an in vivo micronucleus test. In a mouse lymphoma assay, no activity was found with metabolic activation; however, a weak, but significant activity was observed in the absence of such activation.

In studies in human volunteers, there was no evidence of the emergence of cross-resistance to therapeutically important antibiotics but volunteers given oral doses of 20 mg of tylosin daily for 6 months showed an increase in the number of resistant streptococci. The Committee concluded that additional studies showing no microbiological effects in two individuals at doses up to 5 mg per person per day were inadequate to establish a NOEL. In addition, no suitable in vitro data were available to establish a NOEL with respect to the microbiological risk for humans.

Because of the deficiencies in the toxicological and microbiological data, the Committee was not able to establish an ADI.

Residue data

A residue-depletion study was carried out in pigs given radiolabelled tylosin. Three male pigs weighing 66-69 kg were fed a ration containing 110 mg/kg of [14C]tylosin twice daily for 4 days, resulting in a dose of approximately 3.2 mg per kg of body weight. The results showed that total residues of tylosin following oral dosing were greatest in liver and kidney within 4 hours of the last treatment.

Residue-depletion studies with unlabelled drug were performed in cattle, pigs, chickens, and turkeys by different routes of administration, including orally in water and feed, and intramuscular injection.

The studies in which unlabelled tylosin was given to pigs and cattle showed that the tissue in which the highest residue levels occur depends on the method of administration. For injectable forms of tylosin, and excluding the injection site, residue concentrations were highest in kidney and decreased most slowly in this organ, which indicated that it might be the most suitable target tissue. In contrast, for oral dosage forms, liver contained the highest residue concentrations, and might therefore be the most appropriate target tissue when the drug is given orally.

Residue studies in milk cows and laying hens indicate that tylosin passes into milk and eggs. In a group of five cows, following intramuscular injection of 17.6 mg per kg of body weight per day of tylosin for 5 days, the residues in milk (see Table 14) ranged from a mean of 0.75 mg/l at zero hours to less than 0.1 mg/l at 84 hours, as detected by a microbiological method using Sarcina lutea as test organism (limit of detection 0.025 mg/l).

In general, oral dosing of animals resulted in lower tissue residues than injection; orally administered tylosin used for production applications should therefore require a shorter withdrawal time than drug injected for
Table 14
Tylosin residues in cows' milk following intramuscular injection of 17.6 mg per kg of body weight per day for 5 days, as determined by microbiological assay

<table>
<thead>
<tr>
<th>Sampling time (hours)</th>
<th>Mean ± SD (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.75 ± 0.45</td>
</tr>
<tr>
<td>48</td>
<td>0.35 ± 0.21</td>
</tr>
<tr>
<td>72</td>
<td>0.14 ± 0.08</td>
</tr>
<tr>
<td>84</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>96</td>
<td>0.05 ± 0.08</td>
</tr>
<tr>
<td>108-144</td>
<td>No activity</td>
</tr>
</tbody>
</table>

therapeutic purposes. Residues were undetectable by HPLC in poultry tissues after oral dosing, even at zero withdrawal time. The sensitivity of the HPLC methods used was 50 μg/kg.

Although tylosin is extensively metabolized, no single metabolite appears to be present in a greater concentration in tissue than the parent compound, which suggests that the latter should serve as the marker residue. Preliminary studies in pigs showed the tylosin concentration in liver to be approximately 5% of the total extractable residues. Only 60% of total radioactivity was extractable from liver.

Microbiological methods of analysis would be sensitive to any microbiologically active metabolite that might result from the metabolism of tylosin in the animal. Thus the microbiological methods may detect more tissue residue than a chemical method for the determination of tylosin alone. However, microbiological methods, which have quantification limits of 0.1 mg/kg, are not as sensitive as the HPLC method for tylosin in tissue, which has a sensitivity of 0.05 mg/kg.

Until radiolabel studies to determine total residues and investigate metabolism are conducted in all the species concerned, it is difficult to interpret the many studies that show little or no tylosin present as residues.

The Committee was not able to set an MRL because no ADI was established. Before reviewing the compound again, the Committee would wish to have the following:

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

3.4 Tranquillizing agents

The tranquillizing agents considered by the Committee have several points in common. All are old compounds in their class, for all of them the data available to the Committee were inadequate in certain respects, all are often used shortly before slaughter in pigs, and all leave residues in edible tissues. Furthermore, administration by injection is known to create a local area of high concentration of the drug which, in part, is likely to be present at the time of slaughter and, if in edible tissue, is a potential hazard to the consumer.

The Committee therefore advises against the use of these drugs for any purpose in the immediate pre-slaughter period, especially when given by injection into the tissues.

3.4.1 Azaperone

Azaperone had not been previously reviewed by the Committee. It is a butyrophenone neuroleptic tranquilizer for use in pigs.

Toxicological data

A range of studies on azaperone was submitted for assessment, including data on kinetics and metabolism, acute toxicity, short-term and long-term toxicity, developmental toxicity, and genotoxicity. Most of the studies were carried out in the 1970s and the standard of testing and reporting varied widely.

The kinetic studies with azaperone were insufficient to determine the extent of absorption from the gastrointestinal tract. However, by comparison with the excretion pattern after parenteral dosing, it was estimated that absorption after oral dosing was probably high. Distribution within the body in rats was extensive, and excretion was primarily in the faeces (81%), with lesser amounts in urine (16%). Azaperone is extensively and rapidly metabolized (see page 43). Two metabolites were found in the pig but not in the rat. However, these compounds are devoid of significant pharmacological activity, and therefore do not affect the suitability of the rat as a model for toxicological testing.

Azaperone was moderately toxic in acute toxicity studies in mice, rats, guinea-pigs, and dogs. Most signs of intoxication reflected exaggerated pharmacological activity of azaperone in the central nervous system. A
battery of pharmacological studies indicated that azaperone possesses potent anti-α-adrenergic activity, but these data were inadequate for use in determining a NOEL as the drug was almost always given by the subcutaneous route.

Short- and long-term toxicity studies were carried out in rats and dogs. Dose-related sedation was the major effect in both species and was observed at all treatment levels. Minor hepatotoxicity was observed at doses at and above 30 mg per kg of body weight per day in rats and 5 mg per kg of body weight per day in dogs. In rats only, brain weight was consistently increased at 30 mg per kg of body weight per day, but in the absence of any pathological change this observation could not be explained.

There were pathological changes in the pituitary and sex organs, particularly in rats; these were typical of neuroleptic agents. It has been postulated that the primary effect is pharmacological and is caused by the blocking of dopamine receptors in the hypothalamus or pituitary, resulting in increased prolactin and decreased gonadotrophin secretion. While this could account for the observed slight stimulation of the pituitary and mammary glands and the quiescence of the female reproductive tract, direct evidence for this mechanism was lacking. The effects on the reproductive organs were slight, in line with the relatively weak anti-dopamine activity of azaperone. In dogs such effects were observed only after dosing for 24 months with 1.25 and 5 mg per kg of body weight per day but not at 20 mg per kg of body weight per day. In rats, effects were noted after 3, 6, and 12 months but not after 18 months, which suggests the possibility of adaptation. When pharmacological effects were excluded, the NOELs were 1.25 mg per kg of body weight per day in dogs and 8 mg per kg of body weight per day in rats.

The Committee noted that the carcinogenic potential of azaperone had not been adequately investigated. The only study in which lifetime exposure was approached was an 18-month study in rats. However, the duration of dosing was too short and the small group size (only ten rats of each sex) was inadequate to determine treatment-related tumour incidences satisfactorily.

Frame-shift mutations in Salmonella typhimurium strains were seen for azaperone and three metabolites in a series of studies carried out by one group of investigators. However, reversion rates were only 2-3 times those in controls; there was no dose-response relationship, and high doses in the presence of rat liver microsomes were required. This weak response was not reproduced by a second group of investigators using the same bacterial strains. Genotoxicity was absent in the micronucleus and dominant lethal tests in vivo, suggesting that azaperone has low potential for genetic damage.

In a three-generation study in rats, survival of the pups was reduced during lactation in one generation at the highest dose of 40 mg per kg of body
weight per day. There were no adverse effects on other reproduction parameters. It was recognized, however, that an unconventional methodology was used in this study in that males were left untreated and females were dosed on gestation days 6-15 only. The study was considered to be inadequate to enable the potential for effects on reproduction and fertility to be fully assessed.

Embryotoxicity and teratogenicity were examined in mice, rats, golden hamsters, and rabbits. Fetal abnormalities were not observed in any species. Administration of azaperone during the gestation period resulted in embryotoxicity in mice at or above 10 mg per kg of body weight per day and rats at 40 mg per kg of body weight per day. Maternal toxicity and fetotoxicity, in the form of delayed ossification of metatarsals and metacarpals in mice and golden hamsters and reduced fetal weight in rats, were noted at 40 mg per kg of body weight per day. In a perinatal and postnatal study in rats, the survival of pups during the lactation period was reduced at 40 mg per kg of body weight per day.

Human psychotic patients treated with up to 2 mg of azaperone three times a day (about 0.1 mg per kg of body weight per day) showed no clinical effects. At doses of 2.5 mg given three times daily (about 0.125 mg per kg of body weight per day) and above there was dose-related sedation, and at 20 mg three times daily (about 1 mg per kg of body weight per day), dizziness. Haematological and blood-chemistry parameters were not affected at any dose.

There were no effects apart from sedation at 1.25 mg per kg of body weight per day in a 24-month dog study and at 8 mg per kg of body weight per day in an 18-month rat study. There was no NOEL for pharmacological activity in the animals used in the toxicological studies. However, the study in human subjects provided additional information. The NOEL for sedation was 2 mg given three times a day. Since the human subjects were given azaperone in divided doses and it is unclear whether the doses were additive over the course of the day, the NOEL for sedation in humans was taken to be about 0.03 mg per kg of body weight.

In view of the absence of adequate carcinogenicity and reproduction studies and the weak mutagenicity findings in bacteria, the Committee could not establish an ADI. The Committee was aware of data on the tumorigenic potential of other butyrophenone neuroleptic agents, but considered that the structural differences between them were sufficient to preclude the use of this information to support a temporary ADI for azaperone.

Residue data
The Committee considered radiotracer and metabolic studies, together with studies of residue depletion in the edible tissues of pigs following treatment with doses of azaperone normally associated with good veterinary practice.
Metabolic studies on azaperone in pigs revealed at least 11 metabolites. The three major metabolic pathways elucidated primarily through in vitro studies were: (1) reduction of the ketone to yield azaperol (11%) and other reduced compounds; (2) oxidative N-dearylation (17%); and (3) hydroxylation of the pyridine ring (12%). These primary pathways were also observed in the rat but quantitative differences between the two species were observed. In several studies, the amounts and percentages of parent azaperone and azaperol in the total residue were determined.

A number of residue-depletion studies, including two in which radiolabelling was used, were available. After administration of 1 mg/kg of [3H]azaperone intramuscularly to pigs, 2.8%, 4.0%, 5.4% and 5.3% of unchanged drug was present in kidney and 4.9%, 6.3%, 12.8% and 5.2% in liver at 4, 8, 16, and 24 hours, respectively. The total radioactivity decreased rapidly, and by 16 hours the concentration was very low in all tissues, with the exception of the lung, kidney, and liver. At 24 hours after dosing, the highest concentration of residue (0.23 mg/kg) was in liver, while that in muscle was very low throughout the study; kidney initially had the highest total residue. The main excretory route was via the urine.

In a second residue-depletion study, eight pigs were given single intramuscular injections of 4 mg/kg of [3H]azaperone in the left ham. The radiolabel was at the 2-position in the fluorophenyl ring. Two animals were slaughtered at each of four withdrawal times. Tissues were collected and residue levels determined (see Table 15). The amounts of unchanged drug and azaperol in liver and kidney are given in Table 16.

The results of the depletion studies indicate that, at a very short withdrawal time, kidney would be the most appropriate target tissue and azaperol would be present at much higher concentrations than the parent drug. At 24 hours after dosing, liver would be more suitable as the target tissue and azaperone or azaperol could serve as a marker residue. A series of other residue-depletion studies gave similar results.

In pharmacological experiments designed to compare azaperone with azaperone, doses ranging from 1.6 to 200 mg/kg were given to male Swiss mice by intraperitoneal injection. The results demonstrated that azaperol

<table>
<thead>
<tr>
<th>Withdrawal time (hours)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
<th>Skin</th>
<th>Muscle</th>
<th>Injection site</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3.674</td>
<td>11.019</td>
<td>1.217</td>
<td>1.324</td>
<td>0.588</td>
<td>173.9</td>
<td>1.650</td>
</tr>
<tr>
<td>24</td>
<td>0.698</td>
<td>0.625</td>
<td>0.166</td>
<td>0.263</td>
<td>0.041</td>
<td>60.4</td>
<td>0.088</td>
</tr>
<tr>
<td>48</td>
<td>0.441</td>
<td>0.204</td>
<td>0.071</td>
<td>0.064</td>
<td>0.020</td>
<td>44.4</td>
<td>0.044</td>
</tr>
<tr>
<td>72</td>
<td>0.228</td>
<td>0.124</td>
<td>0.104</td>
<td>0.037</td>
<td>0.013</td>
<td>5.8</td>
<td>0.031</td>
</tr>
</tbody>
</table>
Table 16
Total residue (TR), azaperone (AZ), and azaperol (AZOL) (as mg/kg [3H]azaperone equivalents) in pigs given a single dose of 4 mg per kg of body weight intramuscularlya

<table>
<thead>
<tr>
<th>Withdrawal time (hours)</th>
<th>Liver</th>
<th></th>
<th></th>
<th></th>
<th>Kidney</th>
<th></th>
<th></th>
<th></th>
</tr>
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<td>0.009 (3.9)</td>
<td>0.124</td>
<td>0.005 (4.0)</td>
<td>0.034 (27.4)b</td>
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</table>

a Values in parentheses are the percentages of the metabolite in the total residue.
b This value contains an unexpectedly high result for one of the animals.

was less potent in terms of pharmacological effects than azaperone for all the parameters studied. Azaperone was 4-30 times as potent as azaperol.

Methods of analysis
The analytical methods available for use in determining residues of azaperone and azaperol in pig tissues include gas-liquid chromatography, thin-layer chromatography, HPLC, and gas chromatography/mass spectrometry. Some of them, and especially HPLC, have been developed as part of general screening methods for multi-tranquillizer residue detection. The limit of detection is 50 μg/kg for thin-layer chromatography, 1 μg/kg (azaperone) and 2 μg/kg (azaperol) for HPLC, 1 μg/kg for gas-liquid chromatography, and 15 μg/kg for gas chromatography/mass spectrometry. These methods are therefore sensitive enough to detect the residues of interest.

Maximum Residue Limits
The Committee could not recommend MRLs as no ADI was established.

Before reviewing the compound again, the Committee would wish to have the following:

1. Additional data from genotoxicity studies, which should include:
   (a) a study with the Salmonella typhimurium strains that were reported by one laboratory to be sensitive to azaperone and some of its metabolites; and
   (b) studies with cultured mammalian cells in which a variety of effects are investigated, including chromosomal aberrations and the induction of mutations.

   The results of these studies would determine whether further data are required.

2. Studies from which a NOEL for pharmacological effects in humans could be derived.
3. A justification for the protocol adopted in the reproduction study in rats that was submitted, and in particular for the very limited dosing regimen used in the females and the failure to dose the males.
4. Studies on the concentrations of residues of azaperone and azaperol in both muscle and fat of pigs treated with azaperone over a 3-day period.

3.4.2 Chlorpromazine

Chlorpromazine had not previously been evaluated by the Committee. It is used in human medicine in the therapy of psychiatric diseases and in veterinary medicine as a tranquilizer and antiemetic agent.

Chlorpromazine has a broad spectrum of pharmacological activity. It produces behavioural changes and blocks many cell-membrane receptors, notably those for dopamine and norepinephrine. Besides its tranquilizing and sedative actions, it has a number of other pharmacological effects and shows synergism with other classes of central nervous system depressants. Chlorpromazine appears to be variably absorbed but is metabolized in the gut as well as in the liver, where it can accelerate its own metabolism or conjugation. After being absorbed, the drug is widely distributed in the body and its lipophilicity allows it to achieve a high enough intra-membrane concentration to influence the stability or fluidity of cell membranes. In the blood, more than 90% of the drug is bound to plasma proteins. It is metabolized by oxidation, demethylation, and hydroxylation, together with conjugation with glucuronic acid, leading to the formation of a sulfoxide, which was found to possess about one-eighth of the sedative action of the parent drug in the dog. N-Oxide metabolites, on the other hand, undergo significant reduction in a number of species including humans to produce the parent compound again. In humans, chlorpromazine and its metabolites can be detected in urine for 6-18 months after termination of treatment.

Although the drug was introduced into clinical use in the 1950s, and a number of papers on it have been published since, there was a general lack of relevant toxicological data for evaluation.

The intravenous LD₅₀ (median lethal dose) of chlorpromazine was 20, 23, 16, and 30 mg per kg of body weight in mice, rats, rabbits, and dogs respectively.

Data from short-term, long-term, and carcinogenicity studies were not available to the Committee. Limited recent studies suggest that chlorpromazine may be genotoxic, as shown by microbial genotoxicity tests and by tests in human lymphocytes in culture. In addition, it has been established that certain reactive metabolic intermediates are capable of binding to macromolecules, including DNA.

The Committee noted that there were a number of published reports, often containing contradictory results, on the effects of chlorpromazine on reproduction and fetal development in experimental animals and on its
behavioural effects on pups whose mothers had been treated during fetal development. While the design of most of these studies makes them inappropriate for evaluation, the concerns to which they give rise cannot be ignored.

Since chlorpromazine has been in use for such a long period of time a number of published reports are available on the toxicity and side-effects of the drug in humans. Therapeutic doses may cause a number of side-effects in the circulatory and nervous systems, and adverse effects on blood cells, the skin, and the eye. Interference with human pituitary and gonadal function results in galactorrhea and amenorrhea.

In view of the lack of relevant toxicological data, the long-term persistence of chlorpromazine in humans, the spectrum of additional effects of the drug, and the probability that even small doses can cause behavioural change, the Committee was unable to establish an ADI. Furthermore, the Committee suggested that chlorpromazine should not be used in food-producing animals.

3.4.3 Propionylpromazine

This is the first occasion on which propionylpromazine has been reviewed by the Committee. The drug is a phenothiazine tranquilizer that has been used in all the domesticated animals.

Toxicological data
The manufacturer was unable to supply the Committee with data on the pharmacology and toxicology of propionylpromazine and the published literature has yielded relatively little information. However, the drug is of known efficacy in clinical use.

Both pigs and horses are able to metabolize propionylpromazine, at least in part, but in neither case was a full distribution, metabolism, elimination, or balance study available. The drug binds extensively to tissue and to proteins, and also accumulates in fatty tissues. In rats and pigs, the drug is able to enter the brain and, in dogs, has been shown to cause minor changes in cerebrospinal fluid and serum. These effects were observed at dose levels as low as 0.3 mg/kg given intravenously. In rats, a reduction in the concentrations of pituitary gonadotrophins has been demonstrated in brain and serum following the daily intraperitoneal injection of propionylpromazine at 1.5 mg/kg for 2 weeks.

The intravenous LD₅₀ of propionylpromazine in mice was 38 mg/kg.

In a 4-week study of groups of 5 female and 5 male rats exposed to propionylpromazine in the diet at 0, 60, 360, and 2160 mg per kg of body weight per day, a decreased rate of weight gain was seen each week in both sexes at the highest dose and in females at 360 mg per kg of body weight per day, which was associated with reduced feed intake. A reduction in ovarian weight in all treated females and a dose-dependent reduction in
thyroid weight in males prevented the establishment of a NOEL in this study. Slight periportal fatty infiltration, which sometimes involved mid-zone and centrilobular hepatocytes, was seen in all treated animals and was greatest in the high-dose group.

No short- or long-term studies, no systematic studies of the effects of the drug on reproduction or of its teratogenicity, mutagenicity, carcinogenicity, or immunotoxicity, and no reports on its use in humans were available to the Committee. However, several studies have reported the presence of propionylpromazine in pig kidneys collected from abattoirs so that human exposure must therefore be presumed.

The absence of information in several major areas of pharmacological and toxicological importance made it impossible for the Committee to establish an ADI for propionylpromazine. This is regrettable because the Committee was aware that propionylpromazine is used in circumstances in which the consumer will be exposed to residues of the drug that may be capable of exerting a pharmacological effect.

Residue data
There were very few data on the pharmacokinetics or metabolism of propionylpromazine. A study in horses showed that the drug was rapidly absorbed after intramuscular injection, peak plasma levels occurring 30 minutes later; the plasma half-life was 5.12 hours. Propionylpromazine was extensively metabolized in the horse; four of the numerous metabolites have been identified in horse urine. No radiometric studies were available.

No studies were available on the disposition of the total residues of propionylpromazine. Two studies were reported on residues of the parent drug at the injection site and in liver and kidney tissues. Pigs were injected intramuscularly with 0.5 mg per kg of body weight, and the residues of propionylpromazine measured in liver and kidney at 2, 8, and 24 hours withdrawal time by thin-layer chromatography. Propionylpromazine concentrations in liver (about 200 μg/kg) differed little at 24 hours from those at 2 or 8 hours after injection, but concentrations in kidney decreased from 215 to 53 μg/kg between 2 and 24 hours withdrawal time. The amount of propionylpromazine at the injection site also decreased from 22 mg at 2 hours to 19 mg at 8 hours and 6 mg (10-20% of the administered dose) at 24 hours withdrawal time. A single value of 863 μg/kg has been recorded at the injection site in a pig 5 days after the intramuscular injection of 0.5 mg/kg of propionylpromazine.

No marker substance can be identified from the above studies because information on the metabolism and total residue concentrations is lacking.

Good thin-layer chromatography methods are available for identifying propionylpromazine in animal tissues. The lower limit of detection is 2 μg/kg, which is sufficient to control the use of the drug for at least a 24-hour withdrawal time and probably longer. Within the European
Economic Community, several countries use HPLC and one country uses gas chromatography to monitor residues of tranquilizers.

The Committee was not able to set an MRI because:

- no ADI was established;
- the residue data were insufficient, in that no depletion study was carried out, and no marker residue or target tissue was identifiable.

The Committee also expressed concern about the high levels of residues at the injection site.

The Committee was unable to recommend the continued use of propionylpromazine in food-producing animals and, before it would consider the compound again, would require a full range of toxicological and residue data, including data from which a dose producing no pharmacological effects in humans could be established.

4. Recommendations

1. In view of the large number of veterinary drugs requiring evaluation, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held annually for this purpose.

2. The Committee recommends that measures be taken to limit the need for drugs used immediately prior to slaughter and thus likely to leave significant levels of residues in food. With regard to pigs, such measures may include improved pre-slaughter handling, transport and lairage, and the elimination of stress-susceptible pigs from production.

3. With respect to residues at sites of parenteral administration, the Committee recommended that implants or injections of long-acting and slowly absorbed substances should preferably be located in tissues that are discarded at slaughter, e.g., in the ears of cattle.

References


Annex 1

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


4. Specifications for identity and purity of food additives (food colours) (Fourth report of the Expert Committee). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, vol. II. Food colours. Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).


13. Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances (Tenth


46. *Specifications for identity and purity of some food additives, including*


67. *Toxicological evaluation of certain food additives and contaminants*. WHO Food


## Annex 2
### Recommendations on compounds on the agenda

<table>
<thead>
<tr>
<th>Substance</th>
<th>Acceptable daily intake (ADI) for humans and other toxicological recommendations</th>
<th>Recommended maximum residue limit (MRL)</th>
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<td>j-adrenoceptor-blocking agent</td>
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| Carazolol                        | 0–0.1 µg per kg of body weight<sup>1</sup>                                      | Muscle and fat (cattle and pigs): 5 µg/kg<sup>2</sup>  
|                                  |                                                                                 | Liver and kidney (cattle and pigs): 30 µg/kg<sup>2</sup> |
| Anthelmintic agents              |                                                                                 |                                        |
| Febantel                         | 0–10 µg per kg of body weight<sup>1</sup>                                       | Muscle, fat, and kidney (cattle, sheep, and pigs): 100 µg/kg<sup>2,3</sup>  
|                                  |                                                                                 | Liver (cattle, sheep, and pigs): 500 µg/kg<sup>2,3</sup>  
|                                  |                                                                                 | Milk (cattle): 100 µg/l<sup>4,5</sup>                  |
| Fenbenzadole                     | 0–25 µg per kg of body weight<sup>1</sup>                                       |                                        |
| Oxofenazide                      | 0–4 µg per kg of body weight<sup>1</sup>                                        |                                        |
| Antimicrobial agents             |                                                                                 |                                        |
| Spiramycin                       | 0–5 µg per kg of body weight<sup>1</sup>                                       | Muscle (cattle and pigs): 50 µg/kg<sup>2</sup>  
|                                  |                                                                                 | Liver (cattle and pigs): 300 µg/kg<sup>2</sup>  
|                                  |                                                                                 | Kidney (cattle and pigs): 200 µg/kg<sup>2</sup>  
|                                  |                                                                                 | Milk (cattle): 150 µg/l<sup>5</sup>                  |
| Sulfadimidine                    | 0–4 µg per kg of body weight<sup>1</sup>                                       | Allocated in 1989<sup>4</sup>                  |
| Tylosin                          | Not allocated<sup>5</sup>                                                       | No MRLs allocated<sup>5</sup>                  |
| Tranquilizers                    |                                                                                 |                                        |
| Azaperone                        | Not allocated<sup>5</sup>                                                       | No MRLs allocated<sup>5</sup>                  |
| Chlorpromazine                   | Not allocated<sup>5</sup>                                                       | No MRLs allocated<sup>6,7</sup>                |
| Propionylpromazine               | Not allocated<sup>5</sup>                                                       | No MRLs allocated<sup>6,7</sup>                |

### Notes to Annex 2

1 Temporary acceptance (see Annex 3).
2 Temporary MRL (see Annex 3).
3 Group MRL for febantel, fenbenzadole, and oxofenazide individually or in combination. The MRL value is the sum of the concentrations of fenbenzadole, oxofenazide, and oxofenazide sulfone, calculated as oxofenazide sulfone equivalents.
5 Insufficient toxicological information was available to establish an ADI (see Annex 3).
6 MRLs were not established because an ADI was not allocated.
7 Insufficient information was available to establish an MRL.
Annex 3
Further toxicological studies and other information required or desired

**β-Adrenoceptor-blocking agent**

*Carazolol*

Results of the following studies are required for evaluation in 1994:

1. A study to define a pharmacological no-effect level in humans.
2. Radiometric studies on the concentrations of carazolol and its metabolites as proportions of the total residue in pigs and cattle over a 24-hour period.
3. Nonradiometric studies on carazolol residues in pigs, using suitable analytical methods, over a 24-hour period.

**Anthelminthic agents**

*Febantel, fenbendazole, and oxfendazole*

Results of the following studies are required for evaluation in 1995:

1. *In vivo* assays for binding to DNA in liver following the oral administration of febantel, fenbendazole, and oxfendazole to rats.
2. Studies designed to explain the activity of fenbendazole and, in particular, 2-amino-5-phenylsulfinyl-2-benzimidazole in the mouse lymphoma *tk*- locus genotoxicity assay.
3. A mouse lymphoma *tk*- locus genotoxicity assay of oxfendazole.
4. Studies that suggest how fenbendazole might induce tumours in female rats at high doses through a nongenotoxic mechanism.
5. Dominant lethal and micronucleus tests on oxfendazole.
6. A teratogenicity study in rabbits using oxfendazole at sufficiently high doses to explore adequately its teratogenic potential in this species.
7. Studies on the total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone), measured as oxfendazole sulfone, in the edible tissues of cattle and sheep and in the milk of cattle over a 28-day withdrawal period after treatment of animals with fenbendazole or oxfendazole. In particular, information is requested on the use of the pelleted form of fenbendazole in cattle and sheep.
8. Studies on the total residues of the above three metabolites, measured as oxfendazole sulfone, in the edible tissues of pigs given fenbendazole and observed over a 7-14-day withdrawal period.
9. Information on the bioavailability of bound residues in liver after administration of febantel to one of the following species: cattle, pigs, or sheep.
10. Development of a suitable method for the determination of total residues of the three metabolites (fenbendazole, oxfendazole, and oxfendazole sulfone, measured as oxfendazole sulfone) in milk.
**Antimicrobial agents**

**Spiramycin**

Results of the following studies are required for evaluation in 1994:

1. *In vivo* studies on the effects of spiramycin on the intestinal flora.
2. Radiometric studies on the concentrations of spiramycin and its metabolites as proportions of the total residue in edible tissues of cattle, pigs and poultry.
3. Studies on the pharmacokinetics of spiramycin residues in the fat of cattle and pigs and the edible tissues of poultry.

**Sulfadimidine**

Results of studies known to have been completed on the effects of sulfadimidine on the thyroid gland in various animal species are required for evaluation in 1993.

**Tylosin**

Before reviewing the compound again, the Committee would wish to have the following:

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

**Tranquilizers**

**Azaperone**

Before reviewing the compound again, the Committee would wish to have the following:

1. Additional data from genotoxicity studies, which should include:
   (a) a study with the *Salmonella typhimurium* strains that were reported by one laboratory to be sensitive to azaperone and some of its metabolites; and
(b) studies with cultured mammalian cells in which a variety of effects are investigated, including chromosomal aberrations and the induction of mutations. The results of these studies would determine whether further data are required.

2. Studies from which a NOEL for pharmacological effects in humans could be derived.

3. A justification for the protocol adopted in the reproduction study in rats that was submitted, and in particular for the very limited dosing regimen used in the females and the failure to dose the males.

4. Studies on the concentrations of residues of azaperone and azaperol in both muscle and fat of pigs treated with azaperone over a 3-day period.
Annex 4

Matters of interest arising from the Fifth Session of the Codex Committee on Residues of Veterinary Drugs in Foods

The Joint FAO/WHO Expert Committee on Food Additives considered several issues raised in the report of the Fifth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (1).

Carbadox

Concern had been expressed by the Codex Committee that MRLs had been recommended for carbadox at the thirty-sixth meeting of the Expert Committee, even though a numerical ADI was not established (2).

The Expert Committee responded that it considered it inappropriate to determine a NOEL and set an ADI for a genotoxic carcinogen. However, the availability of data on the biotransformation of carbadox, the toxicity of its major metabolites, residue depletion, and the limited extractability of the bound residues, and the fact that no metabolites of toxicological concern could be identified in the extracts of bound residues made it possible to recommend MRLs, even though a numerical ADI could not be established.

Safety factors

Questions were raised about the choice of safety factors in the evaluation of several substances at the thirty-fourth meeting of the Expert Committee (3).

For a discussion of the safety factors used by the Committee, section 2.2 of the main text of this report and *Principles for the safety assessment of food additives and contaminants in food* (4) should be consulted.

The specific substances of interest are considered separately below.

*Albendazole*

The Committee’s initial discussion of an ADI centred on the lowest NOEL of 5 mg per kg of body weight per day for teratogenic effects in rats, rabbits, and sheep, with a safety factor of the order of 1000-2000, given the teratogenic effects exhibited by albendazole. However, comprehensive data were available on the metabolism of albendazole and on the teratogenic potential of the major metabolites, which showed that only the parent compound and the sulfoxide derivative were teratogenic. Furthermore, data on the composition and depletion of residues in edible tissues showed that levels of albendazole and the sulfoxide metabolite fell rapidly in residues, and that the major constituent of the residues in animal tissues intended for human consumption was the nonteratogenic sulfone metabolite. In addition, the majority of the residues were nonextractable
and, of such residues, not more than 15% was bioavailable. It therefore seemed inappropriate to use an ADI based on a NOEL for teratogenic effects, with the use of a safety factor of 1000, for the purpose of proposing MRLs, when the residues in food would not constitute a teratogenic hazard for the consumer. A NOEL of 5 mg per kg of body weight per day was reported in a study in dogs and of 5.8 mg per kg of body weight per day in a multigeneration study in rats. The Committee considered that the lowest NOEL (1 mg per kg of body weight per day in a rat fertility study) could not be used because of the large interval between doses; the next highest dose level was 10 mg per kg of body weight per day. The ADI was therefore based on a NOEL of 5 mg per kg of body weight per day, and a standard safety factor of 100.

**Ronidazole**

The original intention of the Committee was to evaluate the four 5-nitroimidazole compounds as a group, but there were considerable variations in the amount of data available for each compound.

Both the antimicrobial and mutagenic properties of 5-nitroimidazole compounds involve reduction of the 5-nitro group, with the formation of a short-lived hydroxylamine derivative that binds covalently to tissue macromolecules. In the case of ronidazole, while there were positive results in bacterial mutagenicity assays, a variety of *in vivo* mammalian systems gave mostly negative results. Ronidazole was therefore considered not to be genotoxic and a NOEL of 5 mg per kg of body weight per day was derived from recent carcinogenicity studies in mice and rats, in which there was an increased occurrence of lung adenomas/carcinomas and benign mammary tumours, respectively. It was noted that the mechanism for these neoplastic effects had not been elucidated. A temporary ADI was therefore established and further information was requested. The safety factor was selected in the light of the results of genotoxicity studies on ronidazole in mammalian systems and of the two recent carcinogenicity studies in which NOELs for carcinogenicity and for other toxicological effects of concern were identified. The selection of the safety factor was also influenced by the lack of mutagenicity of several metabolites of ronidazole.

**Sulfadimidine**

The compound caused thyroid follicular-cell adenomas in mice and adenomas/carcinomas in rats. This effect was considered likely to be the result of perturbation of the thyroid-hypothalamus-pituitary axis, so that a NOEL based on a sensitive indicator (thyroid follicular-cell hyperplasia) of altered thyroid function would be an appropriate basis for establishing an ADI. A safety factor of 500 was used since the evaluation of the carcinogenicity studies submitted to the Committee had not been finalized and studies were being undertaken to elucidate further the effects of sulfadimidine on the thyroid; the high safety factor was also intended to
address the problem of possible haematological and hypersensitivity reactions which, although rare, may sometimes be serious.

**Intake values for milk**

It had been suggested at the Codex Committee session that the intake value of 1.5 litres of milk per day (3) was too high. The Expert Committee considered this issue, and decided to maintain this intake value for the time being.

**Naming of species**

One delegation at the Codex Committee session had stated that use of the term “all species” for MRLs was too general. For a discussion of this issue, see section 2.7 of the main report.

**Definition of the ADI**

A proposed glossary of terms and definitions had been considered by the Codex Committee, including a definition of “Acceptable Daily Intake” (ADI) taken from Principles for the safety assessment of food additives and contaminants in food (4), which is concerned with food additives. The Expert Committee considered that the following, more general, definition would be more appropriate:

*Acceptable Daily Intake (ADI)*: An estimate of the amount of a substance in food or drinking-water, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable health risk (standard human = 60 kg).

**Effect of good practices in the use of veterinary drugs on recommended MRLs**

With regard to procedures used for establishing recommended MRLs, the Expert Committee wished to reconfirm the decision process outlined in section 2.2 of its thirty-sixth report (2).

MRLs recommended by the Joint FAO/WHO Expert Committee on Food Additives are set on the basis of an ADI covering the whole range of biological activities of the compound, including its toxicological, pharmacological, and antimicrobial potential as applicable. The ADI is expressed as a range extending from 0 to an upper limit. This indicates that, when MRLs are set, an effort should be made to reduce the potential exposure of the consumer to residues of veterinary drugs to levels below the upper limit of the ADI.

In accordance with the definition of Maximum Residue Limit for veterinary drugs, as adopted by the Codex Alimentarius Commission and accepted by the Expert Committee (3), the Committee examines on a case-by-case basis the possibility of reducing MRLs so as to be consistent with good practices in the use of veterinary drugs (GPVD). In this context,
the Committee uses the term GPVD as defined by the Codex Committee on Residues of Veterinary Drugs in Foods (5), namely as representing the officially recommended or authorized usage, including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions.

All conditions of use, and in particular species, indications, routes of administration, dosage regimens, and withdrawal times, as well as the availability of routine analytical methods are considered before any reduction in ADI-based MRLs is proposed. In no case, however, could consideration of GPVD mean that intakes of residues at levels significantly above the ADI would be accepted. On the other hand, when setting MRLs, the Committee is very cautious in its consideration of GPVD in order to avoid any hindrance to world harmonization of trade or future approved extended uses of drugs and any interference with competition between products used for the same indication.

It should, however, be noted that, even if consideration of GPVD does not allow any reduction in the MRLs, this will not necessarily mean that the consumer will realistically be exposed to residues of the order of magnitude of those equivalent to the ADI; compliance with appropriately set (i.e., statistically based) withdrawal times will in any case guarantee that the average residue levels in the edible tissues that the Committee considers result in intakes significantly below the upper limit of the ADI.

References

Annex 5

Procedures for evaluating microbiologically active substances

The assessment of the microbiological risk to humans due to residues of antimicrobial drugs in food was addressed at the thirty-sixth meeting of the Committee (Annex 1, reference 9f). At its present meeting, the Committee further considered procedures for use in evaluating the potential effects on the human intestinal flora of microbiologically active substances.

The following three procedures exemplify those which may be used to establish ADIs and/or MRLs, depending on the availability of data:

1. Doses of the antimicrobial substance that are without effect on the intestinal flora of human volunteers, together with appropriate safety factors, may be directly used to calculate ADIs.

2. In the absence of human data, no-effect doses obtained from specifically designed studies using human intestinal flora, such as studies with holoxenic rodents, combined with higher safety factors may serve as a basis for the calculation of ADIs. However, the validity of the particular animal model used must be examined in each case.

3. In the absence of data from in vivo studies, and where justified, results from in vitro experiments using relevant human gut microflora to identify MICs may also be used to establish temporary ADIs and/or MRLs. Data from in vivo studies is nevertheless required for the establishment of full ADIs.

At its present meeting the Committee further considered the third procedure listed above, namely the use of MICs for elaborating ADIs and/or MRLs for substances with antimicrobial activity (see section 3.3.1 of the main report).

It was assumed that, for given combinations of microorganisms and antimicrobial substances, pressure to select resistant mutants would be appreciable only if the concentrations of the antimicrobial substance in the medium were greater than, or equal to, the MIC for a sufficiently long time. It was furthermore concluded that the selection pressure would be highest in the distal part of the digestive tract, where it was to be expected that considerable recondensation of any nonabsorbed and nondegraded antibiotic would occur. Two items of information therefore appeared to be necessary in any MIC-based assessment of the microbiological risk:

1. An estimate of the concentration without microbiological effect on the relevant microorganisms colonizing the distal part of the human intestine.

2. An estimate of the fraction of the ingested amount of the antimicrobial substance available to the bacteria in that part of the intestine.

In the selection of the concentration without microbiological effect for use in subsequent calculations, standard MIC values may frequently provide
too crude an estimate. They may, for example, need to be corrected to take into account pH, bacterial counts, and other relevant factors determining the conditions of growth. If the absence of a microbiological effect on the human intestinal flora is to be ensured, MIC values also need to be corrected to cover the whole range of MICs from individual species.

In the transformation of a concentration without microbiological effect in the intestine into a no-effect dose, the following factors should be considered: (a) the fraction of an orally administered microbiologically active substance that retains its microbiological activity after passage through the digestive tract and is therefore “bioavailable” to the microorganisms in the distal part of the intestine; (b) the estimated magnitude of the daily faecal bolus; and (c) the safety factor to be applied to cover the variability of the above conditions within the human population. The upper limit of the temporary ADI is then calculated from:

\[
\text{Upper limit of temporary ADI (µg per kg of body weight)} = \frac{\text{Concentration without effect on human gut flora (µg/ml)}}{\text{Daily faecal bolus (g)}} \times \frac{\text{Fraction of oral dose}}{\text{Safety factor}} \times \text{Weight of human (60 kg)} \times \text{bioavailable}
\]

A conservative safety margin should be allowed in the above model. It should be used only to establish temporary ADIs because the procedure employed to estimate the concentration without effect on the intestinal flora contains too many uncertainties. This implies that normally a change in the status of the ADI from temporary to full would require not merely a change in the safety factor but rather the use of a different model (see procedure 1 or 2) based on a directly determined no-effect level for the microbiological effects.
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