Evaluation of certain veterinary drug residues in food

Thirty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives

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Geneva, 30 January – 8 February 1989

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

*Toxicological evaluation of certain veterinary drug residues in food.* (To be published as WHO Food Additives Series, No. 25.)

Specifications are issued separately by FAO under the title:

*Residues of some veterinary drugs in animals and foods.* (To be published as an FAO Food and Nutrition Paper.)

**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.
EVALUATION OF CERTAIN
VETERINARY DRUG RESIDUES
IN FOOD

Thirty-fourth Report of the Joint
FAO/WHO Expert Committee on Food Additives

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at WHO Headquarters, Geneva, from 30 January to 8 February 1989. The meeting was opened by Dr J.-P. Jardel, Assistant Director-General, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and of the World Health Organization. Dr Jardel noted that the meeting was the second to be devoted exclusively to drugs whose evaluation had been recommended by the newly formed Codex Committee on Residues of Veterinary Drugs in Foods. The results of these evaluations were intended for use not only by the Codex Alimentarius Commission, but also by governments in establishing their food-control programmes.

Dr Jardel noted with satisfaction that the Codex Committee on Residues of Veterinary Drugs in Foods had endorsed the methodology used by the Joint Committee at its thirty-second meeting for establishing acceptable residue levels, although the same terminology would not be used by the Codex Alimentarius Commission. He also noted that the recommendations made at the thirty-second meeting had now been embodied in the stepwise procedure for the elaboration of Codex Maximum Residue Levels in meat products.

Dr Jardel noted that a number of complex scientific issues that would require the broad range of expertise represented by the participants were on the agenda. One general item of interest was the consideration of the biological impact of veterinary drug residues bound to cellular constituents in animal tissues. He reminded the Committee that both the Codex Alimentarius Commission and many governments were looking to it to provide practical advice on how to proceed on that important issue.
1. INTRODUCTION

In response to the recommendations of the Joint FAO/WHO Expert Consultation held in 1984 (1), one previous meeting of the Joint FAO/WHO Expert Committee on Food Additives has been held to consider veterinary drug residues in food (Annex 1, reference 80). The present meeting was convened in response to the recommendation made at the thirty-second meeting of the Committee that meetings on this subject should be held regularly (Annex 1, reference 80). 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 establish 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;
(b) to evaluate or re-evaluate the safety of residues of certain veterinary drugs;
(c) to consider the biological impact of veterinary drug residues bound to cellular constituents in animal tissues; and
(d) to discuss and provide advice on matters arising from the report of the third session of the Codex Committee on Residues of Veterinary Drugs in Foods (2) (see Annex 2).

2. GENERAL CONSIDERATIONS

2.1 Modification of the agenda

Metronidazole, which was on the agenda, was not evaluated toxicologically because the necessary data were not made available to the Committee.

The topics of food intake estimates and the bioavailability of veterinary drug residues were added to the agenda for consideration.

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 33 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
Sulfamethazine, which was on the agenda, was evaluated under the name sulfadimidine, which is the international nonproprietary name for this substance.

2.2 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 report of the Committee (Annex 1, reference 80), and in the report of the Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs in Foods (1).

2.3 Maximum Residue Levels

The Committee noted the definition of Maximum Residue Level (MRL) proposed at the third session of the Codex Committee on Residues of Veterinary Drugs in Food (see 2, Appendix III) and concluded that it could be improved by deletion of “any” from “without any toxicological hazard” (line 2, paragraph 2), and addition of “and estimated food intakes” to the last line of paragraph 2. It would then read:

Maximum Residue Level (MRL) is the maximum concentration of residue resulting from the use of a veterinary drug (expressed in mg/kg or μg/kg on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognized as acceptable in or on a food. It is based on the type and amount of residue considered to be without toxicological hazard for human health as expressed by the Acceptable Daily Intake (ADI), or on the basis of a temporary ADI that utilizes an additional safety factor. It also takes into account other relevant public health risks as well as food technological aspects and estimated food intakes. When establishing an MRL, consideration is also given to residues that occur in food of plant origin and/or the environment. Furthermore, the MRL may be reduced to be consistent with good practices in the use of veterinary drugs and to the extent that practical analytical methods are available.

The Committee concluded that the definition of Acceptable Residue Level used at its thirty-second meeting (Annex 1, reference 80) and the definition of MRL that it adopted at the present meeting
were essentially the same. However, the Committee noted that, for a given drug, the value calculated for an MRL would depend upon whether the ADI or Good Practice in the Use of Veterinary Drugs\(^1\) was used as the basis for the calculation and whether other factors were taken into account. Separate terms to cover all situations could lead to confusion, so it was agreed that only the term MRL should be used throughout the Committee's report, along with appropriate descriptions of the origins of calculated values. The final result of the Committee's deliberations on a given drug would be termed a "recommended MRL" for each food, which would be forwarded to the Codex Committee on Residues of Veterinary Drugs in Foods for consideration.

2.4 Bound residues

The use of veterinary drugs in food-producing animals can result in residues that are neither extractable from tissues nor readily characterized. Since some of the compounds on the agenda—trenbolone acetate, albendazole and the 5-nitroimidazoles—produce non-extractable residues, the question arose of how the Committee should evaluate the safety of such veterinary drugs. The Committee therefore defined the terms used and then outlined its approach to the evaluation of such compounds from the point of view of human food safety.

2.4.1 Definitions

The "total residues" of a drug in animal-derived food consist of the parent drug, together with all the metabolites and drug-based products that remain in the food after the administration of the drug to food-producing animals. The amount of total residues is generally determined by means of a study using the radiolabelled drug, and is expressed as the parent drug equivalent in mg/kg of the food.

The "extractable residues" are the residues extracted from tissues or biological fluids by means of aqueous acidic or basic media, organic solvents and/or hydrolysis with enzymes (e.g., sulfatase or glucuronidase) to hydrolyse conjugates. The extraction conditions must be such that the compounds of interest are not destroyed.

\(^{1}\) The officially recommended or authorized usage, including withdrawal periods, for veterinary drugs under practical conditions, as approved by national authorities (2).
The "non-extractable residues" are obtained by subtracting the extractable residues from the total residues and comprise:

(i) Residues of the drug incorporated through normal metabolic pathways into endogenous compounds (e.g., amino acids, proteins, nucleic acid). These residues are of no toxicological concern.

(ii) Chemically bound residues derived by the interaction of residues of the parent drug or its metabolites with macromolecules. These residues may be of toxicological concern.

The "bioavailable residues" are the residues that can be shown, by means of an appropriate method (e.g., the Gallo-Torres method (3)), to be absorbed when fed to laboratory animals.

A "marker residue" is a residue whose level decreases in a known relationship to the level of total residues in tissues, eggs or milk. A specific quantitative analytical method for measuring the concentration of the residue with the required sensitivity must be available.

2.4.2 Residue evaluation

The Committee recommended that, in the absence of other information, a bound residue should be considered of no greater toxicological concern than the compound for which the ADI was set. When the total residue in an animal-derived food did not exceed the recommended MRL, the bound residue should not be examined further. If the total residue exceeded the recommended MRL, the Committee recommended that the best information available should be used to evaluate the contribution of the bound residue to the toxicity of the veterinary drug. This information includes, but is not limited to, data on the chemical structure, bioavailability, metabolism and toxicological activity of the bound residues.

For an example of an approach that may be used, see Annex 3.

2.5 Bioavailability

The biological significance of residues of veterinary drugs in foods usually depends on the extent to which those residues are absorbed when the food is ingested. In the absence of relevant residue data, it should be assumed that all of the residue is bioavailable and that its potency is equal to that of the most toxic component of the residue.
The generation of relevant data for assessing bioavailability usually requires feeding trials using whole tissues, tissue extracts, and “extracted” tissues (tissues remaining after the extraction process) derived from food-producing animals given radiolabelled compounds. The most important information obtained from such a feeding study is the proportion of residue that is not bioavailable for the test animal species used as a consumer model. Care must be taken in such studies to ensure that the amount of animal tissue in the diet is kept low enough to avoid digestive disturbances which could affect the accuracy of the experiment.

The results of chemical studies of residues may provide additional data that will be useful in assessing the bioavailability of these compounds. These studies, carried out under a range of extraction conditions, may include the identification and quantification of residue components, including bound residues.

Extraction processes in which an attempt is made to mimic the absorption processes taking place in the gastrointestinal tract are of value but are currently not considered to be of sufficient accuracy to provide reliable data on bioavailability.

Once generated, bioavailability data can be used to assess the toxicological significance of residues. Where total residue intake is less than the ADI, further consideration of bioavailability is not warranted, but where it is higher than the ADI, it is necessary to consider the contribution, in terms of their quantity and potency, made by individual compounds to the overall toxicity of residues. Estimates of the bioavailability of bound residues may be incorporated in the formula given in Annex 3 for calculating the amount of residue of toxicological concern.

Bioavailability studies assist in the assessment of the toxicological impact of residues in foods of animal origin, and drug manufacturers are encouraged to provide data from such studies for evaluation by the Joint FAO/WHO Expert Committee on Food Additives.

2.6 Food factors and intake data

An important factor to be considered in the establishment of MRLs in various edible tissues and other products of animal origin is the amount of the food item consumed. In the past, the Committee has assumed a daily intake of 500 g of meat in calculating the MRL, but accurate food intake data are difficult to obtain. This is particularly true at the international level, where dietary habits are
influenced by factors such as ethnic origin, religion and climate. Moreover, knowledge is lacking on the fate of residues in the course of food processing (e.g., curing, fermentation, pasteurization and sterilization) and in the domestic preparation of food.

In order to protect all segments of the population, it is reasonable to use intake data at the upper limit of the range for individual edible tissues and animal products. At its present meeting, the Committee has therefore used the following daily intake values: 300 g of meat (as muscle tissue), 100 g of liver, 50 g of kidney, 50 g of tissue fat, 100 g of egg and 1.5 l of milk.

Other assumptions and variables are involved in determining MRLs, including safety factors used in establishing ADIs, withdrawal times,1 the contribution of bound residues and the bioavailability of residues. Against this background, the potential errors in estimating food intake are unlikely to be of great significance; for this reason, no great effort should be devoted to further refining food intake estimates.

It should be noted that the Codex Committee on Residues of Veterinary Drugs in Foods is continuing its survey of information on the dietary intake of veterinary drug residues in Member countries of the Codex Alimentarius Commission in order to provide information to the Joint Committee and other interested parties.

3. COMMENTS ON RESIDUES OF SPECIFIC VETERINARY DRUGS

The Committee re-evaluated the safety and residues of one xenobiotic hormonally active growth promoter. It evaluated for the first time the safety and residues of one anthelminthic drug, three antiprotozoal drugs, two antimicrobial sulfonamides and two trypanocides. In addition, general information concerning the residues of a fourth antiprotozoal drug was reviewed, even though corresponding toxicological data were not available. The recommendations made with regard to the compounds on the agenda are given in Annex 4, while details of further toxicological studies and other information required or desired are given in Annex 5.

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1 The withdrawal time after administration of a drug is the time during which animals or animal products should not be harvested (by fishing, milking, slaughtering, egg collection, etc.) for human consumption.
3.1 Anthelminthic drug

3.1.1 Albendazole

This is the first occasion on which albendazole has been reviewed by the Committee. The drug is used as an anthelminthic in humans and other species.

*Toxicological data.* Comprehensive toxicological data on albendazole were submitted, including the results of studies on its metabolism, carcinogenicity, genotoxicity, effects on reproduction and teratogenicity, and of short-term studies.

Pharmacokinetic studies, although not designed to measure the extent of absorption, suggested that about 20–30% of ingested albendazole was absorbed in mice and rats, about 1% in humans and 50% in cattle. In all the species studied, oral dosing produced very low plasma levels of unchanged drug because of rapid first-pass metabolism in the liver. The primary metabolic reactions were the oxidation of the sulfide moiety of albendazole to the sulfoxide and sulfone, followed by cleavage of the carbamate moiety to form the 2-amino sulfone. This last compound was found to be the main residue in the livers of sheep and cattle. The degradation of albendazole followed similar pathways in rats, mice, cattle, sheep and humans.

In a study in mice in which albendazole was administered in the diet for 25 months, anaemia, leukopenia and testicular degeneration were noted at 400 mg per kg of body weight per day. Hepatocellular vacuolation was produced at 100 and 400 mg per kg of body weight per day. The incidence of endometrial stromal polyps was slightly higher than in concurrent controls, but statistical significance was not achieved and all incidences were within the laboratory historical control range. The no-observed-effect level was 25 mg per kg of body weight per day.

In a 28-month study in the rat in which the compound was given in the diet, the highest dose of 20 mg per kg of body weight per day caused mortality, neutropenia, hypercholesterolaemia, testicular degeneration and hepatic fatty metamorphosis. The incidence of endometrial/cervical tumours and histiocytic sarcomas in the skin showed an apparent increase in certain treated groups. However, when compared with those for the controls, these findings were not statistically significantly different and were within the laboratory
historical control range. The no-observed-effect level was 7 mg per kg of body weight per day.

The Committee noted that questions had been raised regarding the statistical analyses of the mouse and rat carcinogenicity studies and the use of historical controls in the examination of the tumour incidence. The studies were therefore reviewed by the Committee and were found to be satisfactory. The statistical analysis of both rodent carcinogenicity studies was also reviewed and found to have been conducted in accordance with currently acceptable procedures.

Albendazole did not produce bacterial mutations, chromosomal aberrations or morphological transformations in cultured mammalian cells. The 2-aminosulfone metabolite did not produce bacterial mutations.

A three-generation reproduction study in which the compound was administered in the diet was conducted in rats. There were no effects on fertility or reproduction indices, the only findings being reductions in pup postnatal survival and growth at 11.6 mg per kg of body weight per day. The no-observed-effect level was 5.8 mg per kg of body weight per day.

Male rats were treated by gavage in a fertility study. Overt toxic effects and testicular hypoplasia were noted at 10 and 30 mg per kg of body weight per day. Fertility indices were not affected. Litter size and weight were reduced at 30 mg per kg of body weight per day, the former effect being probably due to a lower implantation rate. The no-observed-effect level was 1 mg per kg of body weight per day.

In a perinatal and postnatal study in rats in which albendazole was administered by gavage to pregnant dams on days 16 to 20 of gestation, there was decreased survival, low birth weight and depressed growth during suckling of the pups of the group given 40 mg per kg of body weight per day. There was also some evidence of retarded organ development in the offspring of this group. The no-observed-effect level was 20 mg per kg of body weight per day.

In a teratology study in mice in which albendazole was administered by gavage, there were no adverse effects at dose levels up to 30 mg per kg of body weight per day.

Several teratology studies were conducted in the rat in which the compound was administered either by gavage or by dietary exposure. Embryotoxic and fetotoxic effects and external malformations were produced at doses of 8.8 mg per kg of body weight per day and above. Skeletal malformations, in particular limb defects, which were seen at doses of 6.6 mg per kg of body weight per day and above,
constituted the most sensitive indicator of developmental toxicity. The no-observed-effect level was 5 mg per kg of body weight per day. Qualitatively similar findings were obtained with albendazole sulfoxide, when administered in equimolar amounts, while eight other metabolites of albendazole did not show any effects.

In rabbits, the toxic dose to the dams of 30 mg per kg of body weight per day was associated with embryotoxicity and partially or totally missing digits. Fetal growth retardation was observed at doses of 10 mg per kg of body weight per day and above. The no-observed-effect level was 5 mg per kg of body weight per day.

A teratology study was conducted in sheep using a single oral dose of albendazole on day 17 of pregnancy. Premature delivery, fetotoxic effects and postnatal death were produced at 20 mg per kg of body weight and malformations were increased at 15 and 20 mg/kg. The no-observed-effect level was 10 mg per kg of body weight.

Dogs were dosed for six months by oral administration of the compound in capsules. They showed neutropenia at 30 mg per kg of body weight per day and above. At 60 mg per kg of body weight per day there was also anaemia, decreased body, testicular and uterine weights, and bone marrow hypocellularity. The no-observed-effect level in this study was 5 mg per kg of body weight per day.

Albendazole has been used in 80 countries to treat gastrointestinal parasitic infections in humans at a dose of 400 mg per person, and there are a number of published reports on its use. In field trials in Nigeria, an unpublished report noted that 17 nulliparous women aged between 16 and 18 years were inadvertently given a single dose of 400 mg of albendazole during the first trimester of pregnancy without any apparent adverse effects on the mother or the neonate.

Some of the effects observed in general toxicity studies with albendazole may be explained by the ability of benzimidazoles to interfere with tubulin polymerization and thus inhibit spindle formation and mitosis.

The most significant toxicological manifestation resulting from treatment with albendazole was its teratogenic activity, limb defects in the rat being the most sensitive indicator of developmental toxicity.

A no-observed-effect level of 5 mg per kg of body weight per day was reported in several studies in rats, rabbits and dogs. Although for males in the rat fertility study the figure was 1 mg per kg of body weight per day, it was noted that the next highest dose used in the
study was 10 mg per kg of body weight per day. In addition, in the multigeneration reproduction study in rats, there was no effect of albendazole on fertility at 11.6 mg per kg of body weight per day, which was the highest dose used, and the reported no-observed-effect level was 5.8 mg per kg of body weight per day.

An ADI of 0–0.05 mg per kg of body weight was established for albendazole based on a no-observed-effect level of 5 mg per kg of body weight per day and a safety factor of 100, which the Committee chose for this compound after taking into consideration its poor absorption in humans, rapid metabolism, the lack of teratogenic potential of most of its metabolites, the use of the drug in humans and the identity of its residues in food.

Residue data. The Committee considered information on the residues of albendazole in cattle and sheep. The residue concentrations in edible tissues of cattle were highest at one day withdrawal time and fell rapidly thereafter; they were essentially similar for each of three methods of oral administration (capsule, paste, drench).

The residue pattern in cattle at four days or longer withdrawal time was different from that at one day withdrawal time, when 50% of the residues were extractable; in contrast, less than 4% were extractable at longer withdrawal times. The parent drug was present in tissues at one day withdrawal time but only three metabolites (albendazole sulfoxide, albendazole sulfone and 2-amino-benzimidazole sulfone) were detectable at a withdrawal time of six days or longer. The majority of the residues at withdrawal time four days or longer were non-extractable, and it was concluded from rat feeding studies that not more than 15% of such residues were bioavailable. The concentration of total residues was much higher in liver and kidney than in muscle and fat. After administration of a 15 mg/kg dose to calves, the residues at four days withdrawal time were 0.06 mg/kg in muscle, 0.21 mg/kg in fat, 5.98 mg/kg in liver and 2.15 mg/kg in kidney.

Sensitive analytical methods are available for use in measuring the concentration of albendazole and its metabolites. A variety of extraction procedures can be used, followed by high-performance liquid chromatography as a means of final determination for quantification. The lower limit of sensitivity of this analytical procedure is about 0.05 mg/kg. Gas chromatography–mass spectrometry is used for confirmation of albendazole residues.
The proportion of the 2-aminosulfone metabolite in the total residues in bovine liver samples collected at more than four days withdrawal time was constant, amounting to one-fifth of the total residues. The 2-aminosulfone metabolite was also shown to be equal to one-sixth of the total residues in ovine liver sampled 5 and 8 days after oral administration of 7.5 mg of albendazole per kg of body weight. Measurement of this metabolite thus gave a measure of the total residues in the liver of cattle and sheep.

Based on an ADI of 0-0.05 mg/kg, the maximum ADI for a 60-kg person is 3 mg of albendazole parent drug equivalent. For comparison, the maximum daily intake of residues in tissues from calves and cows was estimated from the comprehensive data made available and is shown in Table 1.

Table 1. Estimated maximum daily intake of albendazole residues from calves and cows

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose of albendazole (mg per kg of body weight)</th>
<th>Withdrawal time (days)</th>
<th>Estimated maximum daily intake (mg of parent drug equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Based on total residues</td>
</tr>
<tr>
<td>Calf</td>
<td>20</td>
<td>1</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥6</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥4</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Cow</td>
<td>10</td>
<td>1</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*It is assumed that: 50% of the residues are extractable and bioavailable; 70% of the extractable residues are albendazole and the sulfone metabolite and have a toxicological potency equal to 1; 15% of the bound residues are bioavailable and have a toxicological potency equivalent to that of albendazole.

*Not available.

There is insufficient information on residues in tissues other than liver for sheep and in pig tissues to enable a daily residue intake from the consumption of tissues of these animals to be calculated.

The estimated maximum daily intake of residues is in the range of the ADI at one day withdrawal time and falls below it at withdrawal times of four days or more. With good practice in the use of veterinary drugs, it is unlikely that the consumer would be exposed to unsafe amounts of residues in bovine tissues; a similar conclusion might be drawn from the limited data available for sheep.

The information available to the Committee on the residues of albendazole arising in sheep after dosing with a constant-release
device was incomplete, but initial calculations indicate that the ADI would not be exceeded by a person consuming meat obtained five days or longer following administration of the device. No data were available on residues in meat obtained less than five days following administration of the device.

In a [14C]albendazole study in lactating dairy cows fed at 10 mg per kg of body weight, the concentration of total residues in milk was highest (3.9 mg/l) at the first milking and declined rapidly to less than 0.1 mg/l at the fourth milking and to about 0.01 mg/l at the sixth milking.

Maximum Residue Levels. The following factors were considered by the Committee in recommending MRLs for albendazole:

(a) The concentration of total residues is about 50 times greater in liver and kidney than in either muscle or fat.
(b) The estimated maximum daily intake of residues at four days withdrawal time or longer does not exceed the ADI (see Table 1).
(c) The concentration of total residues at ten days withdrawal time or longer does not exceed 5 mg/kg in liver or kidney or 0.1 mg/kg in muscle or fat.
(d) The residues at six days withdrawal time or longer contain low concentrations of toxic substances; there are no residues of parent drug and only small amounts of the sulfoxide metabolite.
(e) More than 95% of the residues at four days withdrawal time or longer are bound residues, of which less than 15% are bioavailable.
(f) A withdrawal time of ten days is long enough for the drug to be efficacious and is compatible with good animal husbandry practice.
(g) The concentration of residues in milk declines rapidly following drug administration and is less than 0.1 mg/l by the fourth milking.
(h) The amount of residues in 1.5 l of milk obtained at the second milking is 0.8 mg of parent drug equivalent and, when added to the values for meat and offal at four days withdrawal time, the sum, 1.8 mg, is less than the ADI.

An MRL for total residues of albendazole of 0.1 mg/kg for muscle, fat and milk, and 5 mg/kg for liver and kidney is recommended. In practice, the total residue is determined in tissues.
by measuring the residue of the 2-aminosulphone by means of an
exhaustive extraction and liquid chromatographic procedure and
multiplying the result by five for cattle and six for sheep (2-
aminobenzimidazole sulphone accounts for 20% and 17% of the total
residue in liver from cattle and sheep, respectively). A marker residue
substance in milk needs to be identified and further information is
required on the quantitative relationship between the concentration
of the 2-aminobenzimidazole sulphone and total residues in muscle,
kidney and fat of cattle and sheep.

Monographs were prepared on the toxicological and residue data.

3.2 Antiprotozoal drugs

Four 5-nitroimidazole compounds were considered by the
Committee. They are similar in structure, as is evident from Fig. 1,
and they therefore have several properties in common. They are
active against both anaerobic bacteria and protozoal parasites in
humans and other species. The 5-nitro group is essential for their
therapeutic action. Certain toxicological properties are also
common to the nitroimidazoles, notably their ability to induce
mutations in bacterial test systems and increase tumour yields in
laboratory animals. Both antimicrobial and mutagenic activities
involve the reduction of the 5-nitro group with the formation of a

Fig. 1. Structural formulae of the four nitroimidazoles

\[
\begin{align*}
\text{Dimetridazole} & : \begin{array}{c}
\text{O}_2\text{N} \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\end{array} \\
\text{Ronidazole} & : \begin{array}{c}
\text{O}_2\text{N} \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_2\text{O} \text{CONH}_2 \\
\end{array} \\
\text{Metronidazole} & : \begin{array}{c}
\text{O}_2\text{N} \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_2\text{CH}_2\text{OH} \\
\end{array} \\
\text{Ipronicidazole} & : \begin{array}{c}
\text{O}_2\text{N} \quad \text{N} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\end{array}
\end{align*}
\]
short-lived hydroxylamine derivative that can bind covalently to various tissue macromolecules.

Initially, the Committee intended to deal with these substances as a group; however, this was not possible owing to the variation in the amount and quality of the data available. The compounds are therefore evaluated individually below.

3.2.1 Dimetridazole

Dimetridazole had not previously been reviewed by the Committee. It is used for the treatment and prevention of histomoniasis (blackhead) in turkeys and of swine dysentery.

Toxicological data. The toxicological data considered by the Committee included the results of short-term studies in rats and dogs, long-term studies in rats, a teratogenicity study in rabbits and a multigeneration study in rats, and mutagenicity data.

In the short-term toxicity studies, clinical effects on the nervous system were seen when dimetridazole was incorporated into the diets of rats at 500 mg per kg of body weight per day and of dogs at 270 mg per kg of body weight per day. Dose-related testicular atrophy was seen in all treated groups, where the lowest levels of exposure were equivalent to 100 mg per kg of body weight per day for the rat and 90 mg per kg of body weight per day for the dog. No adverse effects were seen in a more recent 90-day study in the dog in which dimetridazole was administered in capsules at doses ranging from 5 to 40 mg per kg of body weight per day.

Maternal toxicity was evident in all treated groups in a teratogenicity study in rabbits to which dimetridazole was administered in capsules at doses of 0–120 mg per kg of body weight per day. There were slight dose-related reductions in fetal weight, significant only at the highest dose, but no evidence of a teratogenic effect.

In a multigeneration study in the rat in which dimetridazole was incorporated at levels of 100 and 2000 mg/kg in the diet, no compound-related effects on reproductive performance were seen and there was no teratogenic effect.

Dimetridazole and its urinary metabolites in the rat gave positive results in mutagenicity tests on strains of Salmonella typhimurium with nitroreductase activity. All of these compounds gave negative results with nitroreductase-deficient strains.
Negative results were obtained in mutagenicity studies with a variety of in vitro and in vivo mammalian systems, including the dominant lethal assay, micronucleus test, gene mutation assay in Chinese hamster ovary cells and test for unscheduled DNA synthesis.

The results of the three long-term rat studies that the Committee reviewed were reported between 1973 and 1977. While meeting the requirements for that period, they were not conducted in accordance with present-day standards for carcinogenicity studies. In the first study, female rats were fed 2000 mg/kg dimetridazole in the diet for 46 weeks, and then received the same diet as the controls for 20 weeks. There was a significantly increased incidence of benign mammary tumours in the treated group. In the second study, rats of both sexes were fed diets containing 0, 100, 400 and 2000 mg/kg dimetridazole for 122 weeks. There was a dose-related increase in the incidence of benign mammary tumours, with an increase in multiplicity (the number of tumours per tumour-bearing animal), in females in the two higher-dose groups. In the third study, rats were fed diets containing 0 or 10 mg/kg dimetridazole for 128 weeks. The small increase in the incidence of mammary tumours in females was not statistically significant.

Because of the lack of mutagenic effect of dimetridazole in in vitro and in vivo mammalian systems, the Committee considered that the mechanism for the production of an increased number of benign mammary tumours in the rat was unlikely to be genotoxic. However, no evidence was submitted to suggest a possible mechanism.

Although a no-observed-effect level of 100 mg/kg in the diet, equal to 4 mg per kg of body weight per day, was reported in the multidose long-term rat study, the Committee could not establish an ADI solely on the basis of this study in the absence of the results of a carcinogenicity study in a second species.

**Residue data.** The Committee considered data on the depletion of residues of dimetridazole from the edible tissue of chickens, turkeys and swine.

The concentration of residues of dimetridazole, as determined by polarography or high-performance liquid chromatography, decreased to less than 0.1 mg/kg in the edible tissues of chickens by one day of withdrawal and to less than 2 μg/kg in the edible tissues of turkeys and swine by two days of withdrawal.
Limited data on total residue depletion were available from six pigs to which a single oral dose of $[^{14}\text{C}]$dimetridazole was administered and which were killed at different times after dosing. At zero withdrawal time, the concentration of total residues was 8.6 $\mu$g/g in muscle, 15.4 $\mu$g/g in liver, 36.1 $\mu$g/g in kidney and 3.6 $\mu$g/g in fat. At seven days withdrawal time, the concentration of total residues was 0.32 $\mu$g/g in muscle, 0.91 $\mu$g/g in liver, 0.81 $\mu$g/g in kidney and 0.37 $\mu$g/g in fat.

Dimetridazole is absorbed from the gastrointestinal tract in both laboratory and target species. About 88% of the administered dose is eliminated from turkeys within three days, whereas about 76% is eliminated from pigs within seven days. In both turkeys and pigs, the predominant metabolite is 2-hydroxymethyl-1-methyl-5-nitroimidazole.

The metabolism of dimetridazole in turkeys and swine has been studied following administration of $[^{14}\text{C}]$dimetridazole. The ring-intact, nitro-containing metabolites identified included 2-hydroxymethyl-1-methyl-5-nitroimidazole, 1-methyl-5-nitroimidazole-2-carboxylic acid and the sulfate and glucuronide conjugates of the former. In attempts to elucidate further the nature of the total residue, radioactivity was found to be associated with cellular macromolecules as well as simpler molecules. The metabolism of dimetridazole leads to reduction of the 5-nitro group, fragmentation of the imidazole ring and formation of covalently bound residues.

Several analytical procedures have been developed for use in monitoring residues of dimetridazole. The early methods relied primarily on polarographic techniques. Recently, a procedure linking high-performance liquid chromatography with electrochemical detection has been developed which can simultaneously determine concentrations of dimetridazole and 2-hydroxymethyl-1-methyl-5-nitroimidazole in swine tissue to about 0.5 $\mu$g/kg.

Before reviewing the compound again, the Committee would wish to see results from the following studies:

1. A long-term study in mice.
2. Studies aimed at investigating the mechanism of tumorigenesis.
3. Adequate studies of total residue depletion in poultry and swine using ring-labelled $[^{14}\text{C}]$dimetridazole.
4. Metabolism studies in swine and poultry that characterize the total/bound residues.

Monographs were prepared on the toxicological and residue data.
3.2.2 Ipronidazole

Ipronidazole had not been previously reviewed by the Committee. The compound is used for the treatment and prevention of histomoniasis in turkeys and dysentery in swine.

Toxicological data. The Committee considered pharmacokinetic data from rats and data from studies on carcinogenicity in mice and rats, mutagenicity, embryotoxicity and teratogenicity in rats and rabbits and reproduction in rats, and from long-term and short-term studies in rats and dogs.

In the rat, after oral administration of [14C]ipronidazole, 27% of the total radioactivity is excreted in urine, 34% in bile and 31% in the faeces. Two hydroxylated metabolites still containing the 5-nitro group have been identified in the faeces of turkeys and rats after oral administration of ipronidazole.

Ipronidazole showed mutagenic properties in bacterial test systems. Because of the inadequate design of studies in mammalian test systems, the Committee could not properly evaluate the genotoxic potential of this drug.

In a carcinogenicity study in Charles River CD1 mice in which ipronidazole was administered in the diet, a significant increase in the incidence of benign proliferative lesions (adenoma and hyperplasia) in the lung was observed at 1000 mg/kg in the diet in both sexes. Although this type of tumour is common in this strain of mice, the number of tumours also exceeded the range reported for the laboratory historical controls. In this study, the no-observed-effect level was 200 mg/kg in the diet, equal to 30 mg per kg of body weight per day for mice.

In a combined carcinogenicity and chronic toxicity study with Sprague-Dawley CFY rats, dietary concentrations of ipronidazole of 0, 20, 200 and 2000 mg/kg were used. Although a high incidence of mammary tumours was seen in all treated female groups (74-96%) and control female rats (84%), the incidence was higher in the high-dose group than in the controls. In addition, mammary tumours appeared sooner and the number of mammary tumours per tumour-bearing rat was higher in the females receiving high doses. The Committee concluded that the results of this study indicated an effect of ipronidazole on mammary tumour formation in female rats in the high-dose group. However, the high incidence of tumours in the control animals, which is common in this rat strain, precluded
the determination of a definite no-observed-effect level. Although the results of this study showed changes in some haematological and clinical biochemical parameters, similar effects were not observed in a 90-day rat study in which higher doses of ipronidazole were used.

In a chronic toxicity study in dogs, which received ipronidazole at 0, 20, 200 or 2000 mg/kg in the diet, decreased body weight, changes in clinical biochemical values, fat depletion and changes in liver and lung weight were observed in the high-dose group; the no-observed-effect level was 200 mg/kg, equal to 5.4 mg per kg of body weight per day.

Studies on embryotoxicity and teratogenicity in rats and rabbits did not reveal any effects at levels of 100 mg per kg of body weight per day for rats or 10 mg per kg of body weight per day for rabbits, which represent the no-observed-effect levels for these studies.

In a three-generation study in rats fed diets containing 0, 20, 200 or 2000 mg/kg of ipronidazole, reduced growth was noted in the highest-dose group. The fertility indices were not affected, although degenerative changes in the testes occurred in one animal in each of the treated groups (each group consisted of 20 females and 10 males). The no-observed-effect level for this study was 200 mg/kg in the diet, equal to 10 mg per kg of body weight per day.

In a 13-week study in which rats were given ipronidazole in the diet, a no-observed-effect level of 80 mg per kg of body weight per day was established. Hepatocellular hypertrophy was observed in male rats at the highest dose level.

In a 13-week study in dogs in which the compound was administered in capsules, all of the dogs in the highest-dose group either died or were taken off treatment. Loss of body weight, dehydration and ataxia were the most prominent signs of toxicity. These clinical signs occurred to a lesser degree in the middle-dose group. The no-observed-effect level was 20 mg per kg of body weight per day in this study.

The Committee was not able to establish an ADI because the rat carcinogenicity study was inadequate to determine a no-effect level for ipronidazole.

**Residue data.** The Committee then considered data on the depletion of residues of ipronidazole from the edible tissues of turkeys and swine treated with ipronidazole at dose levels normally used in good animal husbandry practice. The tissues were assayed
for parent ipronidazole and its primary metabolite, 1-methyl-2-(2'-
hydroxyisopropyl)-5-nitroimidazole, the gas-chromatographic
method used having a lower limit of sensitivity of 2 μg/kg for each
of these compounds. Residues of ipronidazole and the primary
metabolite were not detectable in any of the edible tissues of treated
turkeys and swine by 1-4 days of withdrawal.

Limited data on total residue depletion were available from two
turkeys and one pig treated with a single oral dose of
[14C]ipronidazole and killed five days later. The total residue
concentrations in edible tissues of turkeys were 71.2 μg/kg in muscle,
285.4 μg/kg in liver, 257.7 μg/kg in kidney, 24.8 μg/kg in fat and
92.2 μg/kg in skin and adhering fat. In the pig, total residue
concentrations were 41.1 μg/kg in muscle, 192.7 μg/kg in liver,
189.5 μg/kg in kidney and 20.6 μg/kg in fat.

The only two drug-related compounds identified in the tissues of
turkeys and swine to which ipronidazole was administered were
parent ipronidazole and the 2-hydroxy metabolite. Extractability
studies on the tissues of swine treated with [14C]ipronidazole did not
characterize the total residues.

Bioavailability studies were conducted in rats using tissues from
the aforementioned studies on total residues. Bioavailable residues
amounted to 49% of the total in turkey liver, 68% in turkey muscle
and 49% in swine liver.

An acceptable analytical method, namely gas chromatography
with an electron-capture detector, is available for measuring
ipronidazole and the 2-hydroxy metabolite to 2 μg/kg.

Before reviewing ipronidazole again, the Committee would wish
to see results from the following studies:

1. Adequate in vitro and in vivo genotoxicity studies in mammalian
   systems.
2. A carcinogenicity study in rats to assess the effect of ipronidazole
   on the mammary gland and other tissues.
3. Studies aimed at investigating mechanisms that might explain the
   effect of the drug in increasing the incidence of mammary
tumours in rats and lung tumours in mice.
4. An in vivo metabolism study using ring-labelled ipronidazole in
   the rat. Special emphasis should be placed on the detection of
   metabolites in which the 5-nitro group has been reduced.
5. Adequate studies of total residue depletion in swine and turkeys
6. Metabolism studies in swine and turkeys that characterize the total residues.

Monographs were prepared on the toxicological and residue data.

3.2.3 Metronidazole

Metronidazole was not evaluated toxicologically because the relevant data were not made available to the Committee.

The depletion of residues of metronidazole in food-producing animals has not been studied.

The metabolism of metronidazole in rats, dogs, humans, bacteria and in vitro systems has been investigated. In mammals, six nitro-containing, ring-intact metabolites were identified, namely the sulfate and glucuronide conjugates of metronidazole, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and its glucuronide, 1-(2-hydroxyethyl)-2-carboxyl-5-nitroimidazole and 2-methyl-5-nitroimidazol-1-ylacetic acid. In bacteria and in vitro systems, the fragmentation of metronidazole was observed to lead to many simple, naturally occurring molecules.

Because metronidazole is not approved for use in food-producing animals, no specific methods for the determination of residues of metronidazole in edible tissues have been reported.

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

1. Comprehensive information on its toxicology so that a safety evaluation can be made.
2. The results of studies of total residue depletion in food-producing animals using ring-labelled [14C]metronidazole.
3. The results of metabolism studies in food-producing animals that elucidate the nature of the total residue.

In addition, an analytical procedure needs to be developed for measuring and identifying the residues of metronidazole in the edible tissues of food-producing animals.

A monograph summarizing the residue data was prepared.

3.2.4 Ronidazole

Ronidazole had not been previously evaluated by the Committee. It is used in the control of histomoniasis of turkeys and swine dysentery caused by Treponema hyodysenteriae. The Committee

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considered the results of biochemical and toxicological studies conducted over more than 20 years.

Toxicological data. The toxicological data available to the Committee were derived from studies on the acute toxicity of ronidazole in the mouse, rat and rabbit, its subchronic toxicity in the rat and dog, and its long-term toxicity in the mouse, rat and dog, a three-generation reproduction study in the rat, teratogenicity studies in the mouse, rat and rabbit, and an array of mutagenicity studies both on the parent compound and on some of its metabolites.

Ronidazole is absorbed from the gastrointestinal tract in both laboratory and target species. In studies using radiolabelled ronidazole, the radioactivity has been found to be widely distributed in tissues and eliminated in the urine, faeces and expired air of the animals. The parent compound accounts for part of the urinary excretion but is almost absent from faeces. Ronidazole is rapidly degraded in pig urine and faeces. The ultimate fate of ronidazole metabolites has not been fully determined.

The mutagenic potential of ronidazole was investigated in a range of studies. Positive findings were recorded in bacterial assays with and without metabolic activation, and in the sex-linked recessive lethal test in Drosophila melanogaster. The bone-marrow cytogenetic assay in CF1S mice yielded weakly positive or negative results, while micronucleus tests and a dominant lethal assay were negative.

In addition, a range of postulated and/or identified metabolites of ronidazole and extract of muscle from ronidazole-treated pigs gave negative results in Ames tests. The Committee found these studies helpful in its safety assessment of ronidazole.

The carcinogenicity studies most appropriate for evaluation were an 81-week feeding study in Alderley Park mice and a 104-week feeding study in Charles River CD rats. The data on individual animals were not available to the Committee. The mice received ronidazole in the diet at 0, 5, 10 or 20 mg per kg of body weight per day. The increased occurrence of lung adenoma/carcinoma was statistically significant at 20 mg per kg of body weight per day in males and females. The rats received ronidazole in the diet at 0, 5, 10 or 20 mg per kg of body weight per day, and the increased occurrence of benign mammary tumours was statistically significant in females at 10 and 20 mg per kg of body weight per day and in males at 20 mg per kg of body weight per day. The no-observed-effect level in these studies was 5 mg per kg of body weight per day.
The Committee noted that the mechanism by which ronidazole exerts its dose-dependent carcinogenic effects had not been elucidated.

In the rat carcinogenicity study, it was also noted that testicular atrophy was present in males receiving 20 mg per kg of body weight per day that died between 52 weeks of treatment and the planned end of the trial. In a two-year study in which ronidazole was administered to dogs in capsules to give 10, 20 or 30 mg per kg of body weight per day the same phenomenon was observed, in terms both of a decrease in absolute testicular weight and of the presence of histopathological change. Clinical signs of toxic effects in the central nervous system were observed at all dose levels in this study. In the first year of the study compound-related histopathological changes were found at autopsy in brain tissues from five of seven dogs dosed at 30 mg per kg of body weight per day. A subsequent two-year study in dogs established a no-observed-effect level of 5 mg per kg of body weight per day.

In a three-generation reproduction study in Charles River CD rats, ronidazole was included in the diet at 200, 400 and 800 mg/kg. There were no adverse effects on reproduction nor were compound-related teratogenic effects seen, but the average number of pups per litter was significantly reduced at 800 mg/kg.

In two other studies of Charles River CD rats, no drug-related teratogenic effects were evident in doses up to 200 mg per kg of body weight per day. Maternal weight gain was depressed at 200 mg per kg of body weight per day, while fetal weight was reduced at doses of 100 mg per kg of body weight per day and above. In other studies, CF₁S mice received ronidazole at 50, 100 or 200 mg per kg of body weight per day by gavage and New Zealand rabbits received oral doses of 3, 10 or 30 mg per kg of body weight per day. Despite evidence of maternal toxicity at the highest doses, there was no statistically significant evidence of teratogenicity.

On the basis of a no-observed-effect level of 5 mg per kg of body weight per day and a safety factor of 200, the Committee established a temporary ADI of 0–0.025 mg per kg of body weight. The safety factor was selected by the Committee in the light of the results of genotoxicity studies on ronidazole in mammalian systems and of the two recent carcinogenicity studies in which no-observed-effect levels for carcinogenicity and for other toxicological effects of concern were identified. The selection of safety factor was also influenced by the lack of mutagenicity of several metabolites of ronidazole.
**Residue data.** The Committee then considered data on the depletion of residues of ronidazole from the edible tissues of turkeys and swine. In turkeys given $[^{14}C]$ronidazole at the usual levels in the feed for up to four days, total residue concentrations at zero withdrawal averaged 3 mg/kg in muscle, 4.7 mg/kg in kidney and 4.5 mg/kg in liver. The concentrations fell to 0.26 mg/kg in muscle, 0.14 mg/kg in kidney and 0.05 mg/kg in liver at ten days of withdrawal.

In swine given $[^{14}C]$ronidazole orally at the usual levels for three days, total residue concentrations at zero withdrawal were 8.6 mg/kg in muscle, 12.3 mg/kg in liver, 11.9 mg/kg in kidney and 2.5 mg/kg in fat. At seven days, the concentration of total residues decreased to 0.52 mg/kg in muscle, 1.15 mg/kg in liver, 0.85 mg/kg in kidney and 0.25 mg/kg in fat. Total residues persisted in edible tissues at 42 days, at which time muscle contained 130 µg/kg and liver, kidney and fat 50–60 µg/kg.

The concentration of residues of the parent drug, as determined by a polarographic method of analysis, decreased to less than 2 µg/kg in the edible tissues of swine treated at recommended levels by two days of withdrawal.

The metabolism of ronidazole in turkeys and swine has been studied by the administration of $[^{14}C]$ronidazole. Three ring-intact metabolites were identified in the total residue: 2-hydroxy-methyl-1-methyl-5-nitroimidazole, 1-methyl-2-hydroxymethyl-5-acetamidoimidazole and 1-methyl-2-carbamoylloxymethyl-5-acetamidoimidazole. Radioactivity was found to be associated with cellular macromolecules as well as with simpler molecules. The metabolism of ronidazole leads to the reduction of the 5-nitro group, the fragmentation of the imidazole ring and the formation of covalently bound residues.

The bioavailability of residues has been studied by feeding to rats muscle from a pig killed after seven days of withdrawal. Approximately 48% of the residue was bioavailable.

A combination thin-layer chromatographic and differential pulse polarography method for the determination of ronidazole in the edible tissues of swine has been described; it has a level of sensitivity of 2 µg/kg. 2-Hydroxymethyl-1-methyl-5-nitroimidazole, the major metabolite of ronidazole, is poorly extracted in the initial step; the method is therefore specific for ronidazole.

For a 60-kg person, the permitted daily intake of ronidazole drug equivalents would be 1.5 mg contributed by 500 g of animal-derived
food. The Committee used the available data on total residue depletion in turkeys and swine and the revised food consumption estimates (see section 2.6) to determine when the maximum daily intake of total residues of ronidazole would not exceed this ADI. As shown in Table 2, a one-day withdrawal period in turkeys and a two-

<table>
<thead>
<tr>
<th>Species</th>
<th>Withdrawal time (days)</th>
<th>Estimated maximum daily intake of total residues from all tissues (mg of parent drug equivalent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine</td>
<td>0</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.8</td>
</tr>
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<td>1.4</td>
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<tr>
<td>Turkey</td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

day withdrawal period in swine should ensure that the daily intake of total residues of ronidazole does not exceed the ADI. Data were not available to enable bound residues to be taken into account.

The data on total residue depletion do not provide a good correlation between a marker compound and the total residue. However, the Committee noted that the concentration of residues of ronidazole, as determined by polarographic methods, decreases to below the detection limit (2 μg/kg) by two days of withdrawal in swine. Similar data are not available for turkeys.

The data submitted for the evaluation of ronidazole raised complex issues. Extensive studies were performed by the manufacturer in an attempt to assess the toxicological significance of the bound residues. However, the Committee did not fully consider the data in the context of establishing a relative toxicological potency for these residues. Instead, the toxicological potency was assumed to be equal to that of the parent drug for the purposes of the procedure for estimating the maximum daily intake of residues (see Annex 3). The failure to establish a relative toxicological potency of the bound residues, together with the absence of additional data showing that a relationship exists between a marker compound and the total residue, contributed to the inability of the Committee to establish an MRL for ronidazole.

The Committee will need further discussions on new information provided on the bound residues of ronidazole so that data of this type may be fully applied to the safety evaluation of veterinary
drugs. The Committee welcomes the submission of relevant new data.

The Committee requires the following by 1993:

1. A complete submission, including data on individual animals, of the carcinogenicity study reports.
2. The results of studies aimed at investigating the mechanism of tumorigenesis.

Monographs were prepared on the toxicological and residue data.

3.3 Antimicrobial sulfonamides

3.3.1 Sulfadimidine

Sulfadimidine had not been previously evaluated by the Committee. It is a sulfonamide and is used to treat a variety of bacterial diseases in humans and other species and to promote growth in food-producing animals.

*Toxicological data.* The Committee considered toxicological data from studies on carcinogenicity in rats and mice, embryotoxicity and teratogenicity in rats, reproduction in mice and rats and thyroid function in rats, and from 90-day studies in mice, rats and dogs.

In the carcinogenicity study in mice, thyroid follicular-cell adenomas occurred in male and female mice at a dietary level of 4800 mg/kg. In the corresponding study in rats, thyroid follicular-cell adenomas and adenocarcinomas occurred in females at a dietary level of 2400 mg/kg and in males at a dietary level of 1200 or 2400 mg/kg.

The Committee concluded that the thyroid follicular-cell tumours observed in mice and rats were most probably the result of perturbation of the thyroid–hypothalamus–pituitary axis and that humans would not be at carcinogenic risk if exposure to sulfadimidine was below the no-observed-effect level for a sensitive parameter of thyroid function.

In the carcinogenicity study in mice, the no-observed-effect level for thyroid follicular-cell hypertrophy was 86 mg per kg of body weight per day for females and 68 mg per kg of body weight per day for males. In the corresponding study in rats, the no-observed-effect level for thyroid follicular-cell hyperplasia was 2.4 mg per kg of body weight per day for females and 2.2 mg per kg of body weight per day for males.
In the study on thyroid function in rats, the no-observed-effect level for increased thyroid weight was 30 mg per kg of body weight per day for females and 26 mg per kg of body weight per day for males at 12, 18 and 24 months. In this study, the serum concentrations of triiodothyronine and of thyroid-stimulating hormone were highly variable and were not significantly different either at any dose or at any time. The concentration of thyroxine in the serum was decreased in females given sulfadimidine at 1200 or 2400 mg/kg in the diet for 18 months and in males given 600, 1200 or 2400 mg/kg in the diet for 24 months. However, the decrease did not show a consistent dose–response trend.

The no-observed-effect levels for reproductive and teratogenic effects in the rat were 120 mg per kg of body weight per day, which was the highest dose tested. The no-observed-effect level for reproductive effects in the mouse was 720 mg per kg of body weight per day. In this latter study, a significant decrease in the number of litters and in the number of live pups per litter, a significant decrease in live pup weight, and a significant increase in the proportion of male pups among the live pups per litter were observed at the highest dose level.

The Committee established a temporary ADI of 0–0.004 mg per kg of body weight based on the no-observed-effect level of 2.2 mg per kg of body weight per day for thyroid follicular-cell hyperplasia in male rats and a safety factor of 500. This safety factor was used because the Committee was aware that the evaluation of the carcinogenicity studies had not been finalized and that additional studies were being undertaken to examine further the effects of sulfadimidine on the thyroid gland and the possibility of hypersensitivity reactions (see section 3.3.3).

The Committee was also aware of additional studies in progress on the effect of sulfadimidine on the thyroid in various animal species, and requested that the results of these studies should be submitted by 1991.

Residue data. Since the Committee did not receive any information from the manufacturers of sulfadimidine, it considered the data on residues of the drug made available by the authorities in France and the USA and available in the scientific literature. Important data were provided by a study conducted jointly by the Food and Drug Administration and the Department of Agriculture in the United States in which [14C]sulfadimidine was fed at
110 mg/kg of food to pigs. The dose is that normally used in good animal husbandry practice.

Pharmacokinetic studies indicate that sulfadimidine is rapidly absorbed and excreted in both farm animal species and humans. The drug is eliminated and the concentration of residues decreases more quickly when the drug is injected than when given in the feed or drinking-water.

Sulfadimidine is metabolized in the pig to three major metabolites, namely N-acetylsulfadimidine, N-glucosylsulfadimidine and desaminosulfadimidine. The N-acetyl derivative occurs in farm animals, rodents and humans. Less information is available on the metabolism in other species. The parent drug and the three metabolites account for more than 80% of the extractable residues in the pig.

In the study in which [14C]sulfadimidine was administered to pigs, the extractable residues constituted more than 80% of the total residues at eight hours withdrawal time; however, as withdrawal time increased to two, five and ten days, there was a gradual increase in the percentage of [14C]-labelled residues that were not extractable. At ten days withdrawal time, the non-extractable residues amounted to 38% in muscle, 76% in liver, 65% in kidney and 60% in fat. However, the total residues at that withdrawal time were less than 0.06 mg/kg and most of the free parent drug and metabolites were eliminated from the above-mentioned tissues.

The drug was rapidly eliminated following intramuscular injection or intramammary administration to lactating dairy cows. The concentrations of residues in milk declined rapidly and, after three days withdrawal time, were below 0.1 mg/l; the average concentrations were below 0.05 mg/l.

Sheep were injected intravenously with a single dose of sulfadimidine at 107 mg per kg of body weight. The concentration of residues in muscle, liver, kidney and fat declined rapidly and, after five days withdrawal time, was less than 0.1 mg/kg.

The data from the [14C]sulfadimidine study in pigs were used to calculate the maximum daily intake, based on the food intake values given in section 2.6 and the method of calculation given in Annex 3. The results are shown in Table 3.

An ADI of 0.004 mg/kg would be equivalent to a daily intake of 0.24 mg of sulfadimidine for a 60-kg person. This value is exceeded at eight hours and two days withdrawal time but not at five days withdrawal time or longer. The practice of monitoring tissues for
### Table 3. Estimated maximum daily intake of sulfadimidine residues from pigs

<table>
<thead>
<tr>
<th>Withdrawal time</th>
<th>Estimated maximum daily intake [mg of parent drug equivalent]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Based on total residues</td>
</tr>
<tr>
<td>8 hours</td>
<td>2.43</td>
</tr>
<tr>
<td>2 days</td>
<td>0.78</td>
</tr>
<tr>
<td>5 days</td>
<td>0.17</td>
</tr>
<tr>
<td>10 days</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*From the data obtained in a study in which pigs were given 150 mg of \(^{15}C\)sulfadimidine per kg of feed.

*It is assumed that 90% of the residues are extractable and bioavailable; that 10% of the extractable residues have a toxicological potency equivalent to that of the parent drug; in the absence of bioavailability data, that the toxicological potency of the non-extractable residues is equivalent to that of the parent drug.

*NA = Not available.

0.1 mg/kg sulfadimidine would produce the same safe withdrawal time for the pigs in the study.

It has been found, in other studies in pigs, that the concentration of residues declines more slowly when good farming or feed-manufacturing practices are not followed. Pigs are coprophagic and unless their pens are cleaned after the drug has been withdrawn, it will be recycled. Information made available to the Committee showed how difficult it was to produce a feed containing the correct amount of drug and also how easy it was for drug-free feed to be contaminated in the mixing process. These are both important contributory factors in the large number of violations of legal provisions that occur in several countries with pigs dosed with sulfadimidine.

Poultry were dosed for five days with 1 g or 2 g of sulfadimidine per litre in their drinking-water. The residues of the parent drug in eggs were measured by high-performance liquid chromatography. The maximum concentration of residues was 84 mg/kg on the fifth day of treatment, the concentration remaining at a high level during the first two days after the treatment and then decreasing to less than 0.1 mg/kg at eight days and nine days withdrawal time for the 1 g/l and 2 g/l doses respectively. Such a five-day treatment period followed by a nine-day withdrawal period would be difficult to enforce.

Good analytical methods are available for screening, quantifying and confirming residues of sulfadimidine. Simple quantitative methods include thin-layer chromatography and immunoassay, which are suitable for use at the farm or in small laboratories. High-performance liquid chromatography, thin-layer chromatography, and gas chromatography–mass spectrometry or tandem mass
spectrometry are used for monitoring and confirmation of residues. All the methods have a sensitivity of less than 0.1 mg/kg, which is sufficient for most regulatory purposes. There is a good correlation between the concentration of sulfadimidine in plasma or urine and the level in edible pig tissues (4). The concentration of residues in edible tissues may therefore be estimated before slaughter by using body fluids collected from the live animal.

The recommended MRLs for sulfadimidine are as follows (see also Table 4):

— muscle, liver, kidney and fat:
  300 µg/kg expressed as total residues;
  100 µg/kg expressed as sulfadimidine, the marker residue;
— milk:
  50 µg/kg expressed as total residues;
  25 µg/kg expressed as sulfadimidine;
— eggs:
  300 µg/kg expressed as total residues.

On the basis of these data, it has been estimated that the withdrawal time needed for eggs (see Table 4) is not enforceable.

<table>
<thead>
<tr>
<th>Tissue or food</th>
<th>Daily food intake</th>
<th>Recommended MRL as total residues (µg/kg)</th>
<th>Residue intake* (µg)</th>
<th>Withdrawal time (days) to comply with MRL</th>
<th>% Sulfadimidine at 5 days withdrawal time</th>
<th>Concentration of sulfadimidine in tissue* (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td>0.3 kg</td>
<td>300</td>
<td>90</td>
<td>5</td>
<td>34</td>
<td>100</td>
</tr>
<tr>
<td>Liver</td>
<td>0.1 kg</td>
<td>300</td>
<td>30</td>
<td>5</td>
<td>18</td>
<td>54</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.05 kg</td>
<td>300</td>
<td>15</td>
<td>5</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>Fat</td>
<td>0.05 kg</td>
<td>300</td>
<td>15</td>
<td>2</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Milk</td>
<td>1.5 l</td>
<td>50</td>
<td>75</td>
<td>1+3†</td>
<td>NA†</td>
<td>NA†</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.1 kg</td>
<td>300</td>
<td>30</td>
<td>8+5*‡</td>
<td>NA‡</td>
<td>−</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255</td>
</tr>
</tbody>
</table>

*Equal to recommended MRL × daily food intake.
*Equal to recommended MRL × percentage of sulfadimidine in total residues. This would be the concentration of sulfadimidine in tissue if total residues were present at the MRL.
†One day withdrawal time after intramuscular administration or three days withdrawal time after intramuscular administration.
‡The percentage of sulfadimidine in total residues was not available to the Committee.
§Eight days withdrawal time plus five days for the duration of treatment.

There is the possibility that skin allergies in subjects hypersensitive to sulfadimidine could be caused by the consumption of eggs containing high concentrations of residues of the drug (see sections 3.3.3 and 4).

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Monographs were prepared on the toxicological and residue data.

3.3.2 *Sulfathiazole*

Sulfathiazole had not been previously evaluated by the Committee. It is effective against a wide range of Gram-positive and Gram-negative microorganisms.

*Toxicological data.* The Committee considered the toxicological data from 90-day studies in rats and dogs.

From the 90-day study in rats, the Committee concluded that the no-observed-effect level was 18 mg per kg of body weight per day, which was the highest dose tested. The Committee also concluded that the no-observed-effect level in the 90-day study in dogs was 6 mg per kg of body weight per day. A biologically significant increase in relative and absolute thyroid weights occurred in females in the highest-dose group.

The Committee did not establish an ADI because of the lack of data on the hormonal effects of sulfathiazole.

In view of the Committee's conclusion, based on the results of studies on sulfadimidine conducted by the United States Government and published data on other sulfonamides, that the mechanism of sulfonamide toxicity involved the thyroid–hypothalamus–pituitary axis, it decided that carcinogenicity and long-term studies on sulfathiazole were not necessary. Instead, the Committee would wish to see the submission of the results of studies designed to assess the effects of sulfathiazole on sensitive parameters of thyroid and pituitary function in rodents. The Committee would also wish to see the submission of the results of studies designed to elucidate the metabolism of sulfathiazole and to identify and quantify the residues of sulfathiazole in food-producing animals by administration of $^{14}$C-labelled sulfathiazole. In addition, data from mammalian genotoxicity studies would be necessary for a full evaluation of sulfathiazole by the Committee.

*Residue data.* The Committee considered published information on relatively old studies on sulfathiazole in which the drug was administered by both the oral and parenteral routes in swine and ruminants.

The pharmacokinetics of sulfathiazole have been well investigated and show that the drug rapidly penetrates and
equilibrates with many extravascular tissues. In a simple pharmacokinetic model, tissue residues can be accurately estimated from plasma and/or urine concentrations of the drug. Sodium sulfathiazole is rapidly absorbed from the gut, metabolized and rapidly excreted in the urine. In ruminants, the disappearance of sulfathiazole from plasma is controlled by the rate of drug absorption rather than that of elimination, a feature which detracts from its suitability as a veterinary antimicrobial agent. Plasma and urine data were consistent with a one-compartment model with an elimination half-life of 1.1 hours. Acetyl sulfathiazole, a small amount of unidentified polar metabolites, and parent drug were found in plasma and urine.

A definitive radiometric study on sulfathiazole in farm animals does not appear to have been carried out. In addition, no bioavailability data were available.

In human studies, in which a 0.5-g oral dose was administered, the excretion of free sulfathiazole, its acetylation, and the excretion of acetyl-sulfathiazole were described in a kinetic model that had previously been shown to hold for sulfafurazole and sulfamethizole.

The analytical methods used initially for sulfathiazole were based on diazotization followed by colorimetry. More recently, methods similar to those used for sulfadimidine, e.g., thin-layer chromatography, high-performance liquid chromatography, gas chromatography–mass spectrometry and mass spectrometry, have been employed.

Sulfathiazole has the same electrostatic properties as sulfadimidine, so that there is a tendency for unmedicated feed to be contaminated during milling and on animal premises. These properties of the drug also make its use for the treatment of foul brood in bees likely to contribute to the contamination of honey with sulfathiazole residues, especially if it is used during the months when honey production is in progress.

To be able to assess the drug properly, the Committee considered that adequate residue and radiometric studies were needed.

Monographs were prepared on the toxicological and residue data.

3.3.3 Effects of sulfonamides on human health

The Committee noted that most sulfonamides were known to produce antithyroid effects in animals. Sulfamethoxazole,
sulfamoxole, sulfadiazine and sulfalene have produced thyroid effects in experimental animals, including rats, mice and dogs (5–9); these generally take the form of increased thyroid weight, hyperplasia, loss of colloid and sometimes hyperplasia of the thyrotroph cells of the anterior pituitary gland (5, 6). Activity appears to be related to the presence of a para-amino-substituted benzene moiety (10). Sulfadimidine possesses such a functional group and produces antithyroid effects in the rat, characterized by hyperplasia and changes in thyroid hormone levels (11, 12). The effects of sulfonamides and other antithyroid compounds on the thyroid have clear thresholds (13); the mechanisms involve the blocking of normal thyroidal iodine metabolism leading to a reduction in thyroxine production, an increase in the secretion of pituitary thyroid-stimulating hormone, and consequent hyperplasia of the thyroid (5, 7). Prolonged feeding of sulfamethoxazole to rats resulted in thyroid carcinoma (6).

There are reports of effects on thyroid function in humans following treatment with sulfonamides. Co-trimoxazole (sulfamethoxazole, 25 mg per kg of body weight per day for ten days) significantly lowered the concentrations of triiodothyronine and bound and free thyroxine in adults. Similar effects were noted with co-trifamole (sulfamoxole, 12 mg per kg of body weight per day for 10 days) (14, 15). When given to a group of 49 patients including six boys (aged 2–19 years) for up to 11 years, sulfamethoxazole (10 mg/day) had no significant effect on thyroid hormones (16). The Committee concluded that thyroid effects were unlikely to occur in humans except at (and above) therapeutic doses.

The Committee noted that there were a number of reports in the literature of other adverse reactions in humans following treatment with sulfonamides. Of these, the most important were haematological effects, including agranulocytosis and aplastic anaemia, but both were extremely rare (17). Reports of adverse reactions suggest that sulfamethoxazole and a sulfamethizole/sulfamethoxypyridazine combination product are the sulfonamides implicated in the induction of aplastic anaemia in the few cases where this has arisen. There are no reports incriminating sulfadimidine (18, 19).

Hypersensitivity reactions, which normally take the form of skin rashes, have been noted in patients undergoing sulfonamide therapy. The onset is usually within a week of the commencement of
treatment but reactions may appear more quickly following prior sulfonamide sensitization (20).

Although there is no evidence from which a threshold dose for these effects can be determined, the Committee considered that, if hypersensitivity reactions occurred as a result of the ingestion of food of animal origin containing sulfadimidine residues, these would not be of major health significance. The Committee recognized that information on hypersensitivity reactions arising from the ingestion of tissues containing sulfonamides would be extremely difficult, if not impossible, to obtain, and it therefore recommended that residue levels be kept as low as practicable.

3.4 Growth promoter

3.4.1 Trenbolone acetate

Trenbolone acetate (TBA) had been previously evaluated at the twenty-sixth, twenty-seventh and thirty-second meetings of the Committee (Annex 1, references 59, 62 and 80).

Toxicological data. At the thirty-second meeting, the Committee had requested data on individual animals from the three hormonal studies in pigs reviewed at that meeting and the results of a 90-day study with orally administered α-trenbolone (α-TBOH). These data and new information available from the literature regarding cell transformation, mutagenicity and DNA-binding were reviewed.

In Syrian hamster embryo fibroblasts, morphological transformation was induced by both α-TBOH and β-TBOH. The neoplastic potential of the transformed cells was examined by injecting them subcutaneously into nude mice, when fibrosarcomas developed at the sites of injection of the β-TBOH-transformed but not the α-TBOH-transformed cells. No indication of cell transformation was obtained in mouse C3H10T1/2 cells. In Syrian hamster embryo cells but not in C3H10T1/2 cells, induction of micronuclei was observed with both α-TBOH and β-TBOH. On the basis of regression analysis, β-TBOH showed a weak increase in revertant count in Salmonella typhimurium (in the absence of metabolic activation), which would generally not be regarded as a positive result. In vitro covalent binding of β-TBOH was observed to DNA from S. typhimurium and to calf thymus DNA. In the latter
assay, addition of inactive rat microsomal protein reduced the binding about 20-fold.

However, taking into account the results both of the long-term feeding studies in rats and mice reviewed at the twenty-seventh meeting of the Committee and of the comprehensive battery of short-term tests, the Committee concluded that it was unlikely that TBA was genotoxic.

In accordance with the decisions taken at the thirty-second meeting, the Committee decided to base the evaluation of TBA and its metabolites on their no-hormonal-effect level.

The results of the 90-day study with α-TBOH in rats, which had been requested by the Committee, were reviewed but found unsuitable for establishing a no-hormonal-effect level for the α-epimer.

The Committee re-evaluated the results of three hormonal studies in pigs carried out with TBA, α-TBOH or β-TBOH. The previous no-hormonal-effect levels of 10 μg per kg of body weight per day for β-TBOH and 100 μg per kg of body weight per day for α-TBOH, determined in a study with castrated pigs (Annex 1, reference 87), were confirmed.

In a 14-week study in male and female pigs given TBA in capsules, the no-hormonal-effect level in male pigs was between 5 and 7.5 μg per kg of body weight, based on changes in epididymis weight and in plasma progesterone concentration. In a second 14-week dietary study using TBA in growing pigs, the most sensitive effects observed were changes in serum testosterone and estradiol concentrations and testes weight in male pigs. These effects were dose-related and significant at higher dose levels, but marginal at 0.1 mg/kg in the diet, equal to a dose of TBA ranging from 2 to 3 μg per kg of body weight per day.

A safety factor of 100 was applied to the marginal-effect level of 2 μg per kg of body weight per day for TBA in the 14-week study in pigs, giving an ADI of 0–0.02 μg per kg of body weight for TBA. A marginal-effect level of 2 μg per kg of body weight per day for TBA was supported by the no-hormonal-effect level in monkeys of 2 μg per kg of body weight per day for β-TBOH.

At the thirty-second meeting of the Committee (Annex 1, reference 80), it was concluded from a study in pigs that α-TBOH had one-tenth of the hormonal activity of β-TBOH.
Residue data. The data obtained from tissue-residue studies in which TBA was administered to heifers and TBA in combination with estradiol-17β was administered to steers, requested at the thirty-second meeting of the Committee, were submitted, together with data from residue studies in heifers and steers in which each animal received a second implant 60 days after the initial implantation.

The ADI of 0.02 μg per kg of body weight results in a permissible consumption level of residues of TBA of 1.2 μg for a 60-kg person. The Committee estimated the maximum daily intake of residues on the basis of the food intake data given in section 2.6 and two different assumptions: (1) if all the bioavailable bound residues were β-TBOH, the estimated maximum daily intake of total residues would be 1.3 μg of parent drug equivalent; (2) if the bound, bioavailable residues in liver and kidney were α-TBOH and those in muscle were β-TBOH, the value would be 0.70 μg of parent drug equivalent (see Annex 3). Both of these values are in the same range as the ADI.

The Committee recommended MRLs for β-TBOH in muscle and α-TBOH in liver of 2 μg/kg and 10 μg/kg respectively. These MRLs are based on the average residue levels for β-TBOH and α-TBOH, and their variability (the mean value plus three standard deviations) in muscle and liver of heifers at 15–30 days after implantation of 300 mg of TBA. These MRLs are not likely to be exceeded with good practice in the use of veterinary drugs.

Conservative estimates using these MRLs and the daily intake values for edible tissues given in section 2.6 indicate that the ADI for TBA of 0.02 μg per kg of body weight should not be exceeded at any time after implantation of the drug. The maximum concentrations of residues occur at 15–30 days after implantation and are below the recommended MRLs; concentrations will be even lower at the usual withdrawal time of 60 days.

A combination radioimmunoassay–high-performance liquid chromatographic method is available to measure the recommended MRLs reliably in liver and muscle. The Committee was aware of ongoing work to improve the current methods, especially with respect to the development of a mass spectrometric method to confirm TBA.

Monographs were prepared on the toxicological and residue data.
3.5 Trypanocides

3.5.1 Diminazene

Diminazene had not been previously evaluated by the Committee. The compound is widely used as a trypanocide in animals. The Committee considered residue and metabolic data and limited data obtained from genotoxicity, acute and repeated-dose studies. Data from studies in rats indicated that diminazene was absorbed after oral administration but there were no comparable data from other species, including humans.

Toxicological data. No carcinogenicity studies on diminazene and only two conventional mutagenicity studies were available. Trypanosome kinetoplast studies suggested an interaction of diminazene with DNA but the relevance to genotoxicity was unknown; the substance was not an intercalating agent. Diminazene induced respiration-deficient petite mutations in yeast but gave negative results in the mouse micronucleus test. No teratology or other studies were available for use in assessing the reproductive toxicity of the compound. Acute toxicity studies in mice showed some evidence of effects on the central nervous system at high doses. In dogs, cattle and donkeys, intramuscular doses of 7–35 mg per kg of body weight of diminazene produced clinical effects on the nervous system, and necropsy revealed cerebellar haemorrhages and oedema. Hepatotoxic effects were reported in the dog at a dose of 3.5 mg per kg of body weight, although pre-existing liver disease could not be ruled out. Intramuscular doses of 10–40 mg per kg of body weight were hepatotoxic in camels.

Daily doses of 300–500 mg per kg of body weight of diminazene for up to nine months in the diet of rats produced no signs of toxicity. In dogs, repeated intramuscular or oral administration produced signs of toxic effects in the central nervous system, which were confirmed at necropsy by the presence of cerebellar lesions. Feeding diminazene to dogs at 60 mg per kg of body weight per day for nine months produced testicular atrophy; in this study, the no-effect level was 20 mg per kg of body weight per day. There were no acceptable data in humans on which to base any assessment.

The Committee was unable to establish an ADI because the results of adequate studies of toxicity, including studies of carcinogenicity (or genotoxicity), reproduction and teratogenicity, were not available.
Residue data. Radiometric studies with cattle dosed intra-
muscularly with [14C]diminazene at a level of 3.5 mg per kg of body
weight resulted in residue levels of 75, 55 and 2.5 mg/kg in liver,
kidney and muscle, respectively, at a withdrawal time of seven days.
These residue levels decreased to 24, 12 and 1 mg/kg at 20 days
withdrawal time. In a similar study using a colorimetric method of
analysis similar residue levels were observed, even though the dose
of diminazene was 8 mg per kg of body weight. This would indicate
extensive metabolism of diminazene.

The excretion of diminazene in cow’s milk has been investigated
after administration of a dose of 3.5 mg per kg of body weight. The
highest milk levels were found six hours after dosing (0.2–0.5 mg/
kg). The levels were below the limit of detection (0.07 mg/kg)
48 hours after dosing.

The intramuscular use of diminazene in food-producing animals
results in significant residue levels in edible tissues, but these do not
persist. Further information on residue levels and available methods
for measuring milk and tissue residues of diminazene may be needed
if the drug is considered for further evaluation.

The Committee recognized the importance of diminazene in the
treatment of animal trypanosomiasis, but noted that more toxicity
data would be required for a full evaluation. While appreciating that
the data specified below might still not allow such a full evaluation,
the Committee stated that it would wish to have at least the
following before reviewing the compound again:

1. The results of a teratogenicity study.
2. The results of in vitro and in vivo mutagenicity studies so that the
genotoxicity and carcinogenic potential can be evaluated.
3. The results of studies on residue levels in food-producing animals.
4. Information on available methods for measuring milk and tissue
residues of diminazene.

Monographs were prepared on the toxicological and residue data.

3.5.2 Isometamidium

Isometamidium had not been previously evaluated by the
Committee. The compound is widely used as a trypanocide in
animals. The Committee considered residue and metabolic data and
limited data from genotoxicity, teratogenicity, acute and repeated-
dose studies.
Toxicological data. The drug appeared to be poorly absorbed from the gastrointestinal tract of the rat, about 99% of an oral dose being excreted in the faeces. Similar findings were obtained for homidium (ethidium), a contaminant of the commercial product. In the gastrointestinal tract, isometamidium may be converted into homidium but there are insufficient data on this and there is no information on the formation of any other metabolites. Small amounts of radioactive label were excreted in the milk of cows after intramuscular injection of radiolabelled isometamidium.

No carcinogenicity data were available. Isometamidium was a frame-shift mutagen in Salmonella typhimurium in the presence of metabolic activation, like the closely related contaminant and possible metabolite homidium, which is a known DNA-intercalating agent. Isometamidium was also mutagenic in yeast. In an in vivo cytogenetic assay in the rat, it produced numerical changes including hyperdiploidy and endoreduplication but not structural chromosome abnormalities. It gave negative results in a cell-transformation test.

Both isometamidium and homidium were subjected to teratogenicity tests in the rat and rabbit by the intravenous route. In all studies, doses were administered only on selected days of gestation. Isometamidium produced a weak teratogenic and fetotoxic response in the rat at 2 mg per kg of body weight per day, the highest dose tested, but in the rabbit only a very weak fetotoxic response was observed. The no-observed-effect level in rabbits was 0.25 mg per kg of body weight per day. There was no evidence of fetotoxic effects in the rat at 10 mg per kg of body weight per day and only a weak fetotoxic effect in the rabbit at 4 mg per kg of body weight per day when homidium was tested by the intravenous route. No studies were available on isometamidium given orally, but the poor absorption following administration in this way suggests that any no-observed-effect level would be much higher by this route.

Isometamidium had a low order of acute toxicity when given in water to the rat. Rats given a single dose of 1250 mg per kg of body weight showed signs of toxicity characterized by excess salivation, while one out of five females given 2000 mg per kg of body weight died. Rabbits appeared more susceptible to single oral doses of aqueous isometamidium, deaths occurring at doses at 12.5 mg per kg of body weight and above. It was more toxic in rats by the intravenous and intraperitoneal routes than by the oral route. Homidium appeared less toxic in the rat. There were no adequate
short-term toxicity studies, and no adequate studies of effects on humans.

The Committee noted the poor absorption of orally administered isometamidium in rodents, but also that there was no adequate evidence to suggest that either the compound itself or homidium was similarly absorbed in humans. The Committee was not able to establish an ADI because the results of adequate toxicity studies, including carcinogenicity (or genotoxicity) studies and teratogenicity and short-term studies with oral administration of the drug, were not available, nor was there any information on the nature of the metabolites.

Residue data. There were numerous deficiencies in the residue data available for isometamidium; however, even if those deficiencies were made good, the residue levels detected in edible tissues would not be decreased. Firstly, the specifications for the commercial product indicated a minimum purity of only 70% and, while colorimetric methods might detect the four major impurities in isometamidium, chromatographic and radiometric methods would not. Secondly, there was a lack of the metabolism data necessary to determine whether metabolites contributed significantly to the residue levels. Thirdly, the predominant conditions of use of the drug in normal veterinary practice were unclear. Residue data were provided for both intramuscular and intravenous administration of isometamidium at various use levels.

The available information on calves, dairy cows and goats indicated high levels of residues of isometamidium at the injection site and in the liver and kidney after intramuscular administration. Reported levels at the injection site exceeded 1000 mg/kg several days after drug administration. The kidney and liver residue levels ranged from 2 to 7 mg/kg after several days. More information was needed in order to estimate residue levels in muscle and milk.

It was a matter of particular concern that the half-lives of the residues in the edible tissues might exceed 30 days. Animals receiving isometamidium are therefore likely to be given a second injection of the drug before appreciable depletion of the residues can occur. Further information on residue levels and available methods for measuring tissue residues of isometamidium may be needed if the drug is considered for further evaluation.

The Committee recognized the importance of isometamidium in the treatment of animal trypanosomiasis, but noted that more
toxicity data would be required before a full evaluation could be made. While appreciating that the data specified below might still not allow such a full evaluation, the Committee stated that it would wish to have at least the following before reviewing the compound again:

1. The results of studies designed to indicate the nature of the metabolites of isometamidium in the target species, and to provide an indication of the extent to which they are absorbed in laboratory species after oral administration.
2. The results of adequate mutagenicity studies, so that the genotoxicity and carcinogenic potential can be assessed.
3. The results of a teratogenicity study with oral administration of isometamidium.
4. The results of a 90-day study in rats.
5. The results of studies on residue levels in food-producing animals.
6. Information on available methods for measuring milk and tissue residues of isometamidium.

Monographs were prepared on the toxicological and residue data.

4. FUTURE WORK

1. A working paper should be prepared, for consideration at a future meeting of the Committee, on the possible hazards to human health arising from residues in food of veterinary drugs possessing an allergenic potential.

2. The Committee stressed the importance of examining the possible hazard to human health arising from the ingestion of residues of antimicrobial agents administered to food-producing animals, as noted in section 2.1.3 of the report of the thirty-second meeting (Annex 1, reference 80). Although resistance to such agents was not a major factor in the evaluation of the sulphonamides considered at the present meeting, it is likely to become an issue with other antimicrobial agents. A detailed working paper on this issue should therefore be prepared for consideration at a future meeting of the Committee.

3. During the present meeting, the Committee identified certain difficulties in the assignment of MRLs. Current procedures for determining MRLs for different types of veterinary drugs should therefore be reviewed at a future meeting. Specific reference should
be made to those drugs for which a marker residue cannot be identified.

5. 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 regularly for this purpose.

2. The Committee noted that many of the older and widely used veterinary drugs have never been adequately evaluated because of the lack of suitable toxicological data. There is increasing concern by regulatory authorities about, and public awareness of, the presence of residues of these drugs in food, and the fact that MRLs have not been determined. Industry groups and national authorities should therefore be encouraged to generate the necessary toxicological and residue data for safety assessment.

3. In addition to exposure to drug residues in foods of animal origin, certain groups of individuals, such as farm workers and veterinarians, may be exposed to high concentrations of veterinary drugs during the administration of formulated drugs to food-producing animals. The potential health implications of such exposure should be drawn to the attention of the appropriate authorities as the need arises.

4. The Committee reaffirmed the importance of reliable analytical methods in the regulatory control of veterinary drug residues in food-producing animals. The Committee strongly recommended that increased efforts should be made to validate such methods and to obtain the corresponding validation data for submission to future Committee meetings on veterinary drug residues. Few such data were available for consideration at the present and thirty-second meetings of the Committee.

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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).


26. Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants (Fifteenth report of the Expert


63. Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 18, 1983.
64. Specifications for the identity and purity of certain food additives. FAO Food and Nutrition Paper, No. 28, 1983.


Annex 2

MATTERS ARISING FROM THE THIRD SESSION OF THE CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

The summary and conclusions of the report on the third session of the Codex Committee on Residues of Veterinary Drugs in Foods (1) were reviewed by the Joint FAO/WHO Expert Committee on Food Additives. Among other things, during its deliberations, the Codex Committee:

(a) Advanced the proposed draft of Maximum Residue Levels (MRLs) for chloramphenicol, estradiol-17β, progesterone, testosterone and zeranol to Step 5 of the Codex Alimentarius Commission procedure.

(b) Retained the proposed draft MRL for trenbolone acetate at Step 4 in order to allow the Joint FAO/WHO Expert Committee on Food Additives to re-evaluate the compound at its thirty-fourth meeting, on the basis of additional toxicological data.

(c) Agreed to request the Joint FAO/WHO Expert Committee on Food Additives to review the use of the term "unnecessary" when establishing MRLs, in view of the possible negative implications.

(d) Adopted revised definitions of MRL and "good practices in the use of veterinary drugs" and agreed to forward the draft definitions to the Codex Committee on General Principles for endorsement and to the Codex Alimentarius Commission for adoption (see section 2.3 of the main report).

(e) Agreed to forward proposed procedures for the elaboration and acceptance of Codex Maximum Residue Levels to the Codex Committee on General Principles for endorsement and to the Commission for adoption.

(f) Agreed to continue revision of the priority list of veterinary drugs requiring evaluation through the use of a questionnaire, government comments and the Working Group on Priorities, for discussion at the next session.

(g) Agreed to continue the revision of the working papers on methods of analysis and sampling through government comments and the Working Group on Methods of Analysis and Sampling, for discussion at the next session.

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(h) Agreed that the survey of intake studies should continue through government comments, for discussion at the next session.

The Joint Committee noted the discussions of the Codex Committee (paragraphs 70–71, I) on the use of the term "unnecessary" in connection with the setting of ADIs and acceptable residue levels for endogenous growth promoters in the report of its thirty-second meeting (2). The Joint Committee decided to retain this term and recommended that the Codex Committee should continue to use the footnote in Annex 2 of the thirty-second report (2) in which the reasons for its use are explained.

The Joint Committee also noted that the Codex Committee had drawn attention (paragraph 76, I) to the need to define the terms "tissue" and "muscle" so as to avoid confusion when establishing MRLs. The Joint Committee agreed that the term "tissue" referred to all edible animal tissue, including muscle and by-products, while the term "muscle" referred to muscle tissue only. The Joint Committee recommended that the Codex Committee should include these terms in its proposed glossary.

REFERENCES

CALCULATION OF DAILY INTAKE OF RESIDUES TAKING INTO ACCOUNT DATA ON TOXICOLOGICAL POTENCY AND BIOAVAILABILITY

As part of its evaluation of the safety of veterinary drug residues in food, the Committee may need to estimate the maximum daily intake of residues of a particular drug by a person consuming animal tissues. This Annex describes an approach to making such calculations that allows information on the toxicological potency and bioavailability of residues to be taken into account.

Information required

For the veterinary drug under consideration, the following information is required:

(a) the concentration of total residues, extractable residues and bioavailable residues in muscle, liver, kidney, fat, milk and eggs at a known treatment or withdrawal time;
(b) the chemical identity of the residues;
(c) the biological/toxicological potency of the residues of major metabolites;
(d) the ADI for the compound.

Calculations

The amount of residues expressed as parent drug equivalent is calculated for animal-derived food sampled at selected withdrawal times by using the formula given below. It is recommended that these withdrawal times should be chosen so as to include both the time when the residue concentration is at a maximum and the point when the estimated maximum daily intake of residues falls and remains below the ADI for a 60-kg person (for the reasons for selecting a body weight of 60 kg, see section 2.2.3 of the report of the thirty-third meeting of the Committee (I)). It is recognized that, for some drugs, the maximum daily intake of residues may never exceed the ADI.

The amount of residues of toxicological concern is calculated in both the extractable and the bound fraction in each of 300 g of
muscle, 100 g of liver, 50 g of kidney, 50 g of fat and, where appropriate, 1.51 of milk and 100 g of eggs. The concentration of residues is expressed as the concentration of parent drug equivalents, and is calculated for each animal-derived food from the formula:

\[ \text{Residue} = \text{Free residue} + \text{Bioavailable bound residue} \]

\[ = P_0 + \sum_{n=n_1}^{n_k} (M_n \times A_n) \]

\[ + (\text{Bound residue} \times \text{fraction bioavailable} \times A_b) \]

where:
- \( P_0 \) = amount of parent drug per kg of tissue,
- \( n_1 \ldots n_k \) = different metabolites of the parent drug,
- \( M_n \) = amount of parent drug metabolite \( n \) per kg of tissue,
- \( A_n \) = toxicological potency of \( n \) relative to that of parent drug, and
- \( A_b \) = estimated relative toxicological potency of the metabolites in the bound residue (when no information is available, use \( A_b = 1 \)).

In addition:

Bound residue = total residue – (extractable fraction + endogenous fraction).

Where the endogenous fraction (the fraction of residues incorporated through normal metabolic pathways into endogenous compounds) is not known, it should be given a value equal to zero. The fraction bioavailable is estimated from bioavailability data. Where no such data are available, the fraction should be taken as equal to 1.

Example of calculation of daily intake of residues

The Committee took the following into account in calculating the maximum daily intake of residues of trenbolone acetate derived from cattle, based on the known daily intake values for animal tissues:

(a) 15\% of the non-extractable residues are bioavailable (this is a conservative figure based on the observed bioavailability of 4–12\%).
(b) the bound residues in muscle are all β-TBOH;
(c) the bound residues in liver and kidney are α-TBOH (conclusion based on enterohepatic-circulation data);
(d) the toxicological potency of α-TBOH is 0.1 times that of β-TBOH;
(e) the toxicological potency of β-TBOH is the same as that of TBA;
(f) in heifers, the highest residue levels are observed 15 or 30 days after treatment;
(g) since no data on endogenous residues are available, the endogenous fraction is given a value of zero.

Total TBA equivalents in four tissues at 30 days withdrawal time, calculated on the basis of these considerations, are shown in Table A1. The ADI for TBA is 0-0.02 μg/kg or, for a 60-kg adult, 1.2 μg. Based on the food consumption figures in Table A1, the maximum daily intake of residues is 0.70 μg of TBA equivalent (calculated as the total of column IX). (It should be noted that Table A1 is an example only for the purpose of illustrating the method of calculation.)

REFERENCE


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<table>
<thead>
<tr>
<th>Tissue and weight consumed per day (g)</th>
<th>Total residue (µg/kg)</th>
<th>Free residue</th>
<th>Bound residue (µg [β-TBOH]/TBA equivalent per kg)</th>
<th>Total TBA equivalent per kg of tissue (µg)</th>
<th>Total maximum daily intake of TBA equivalent (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBA (µg/kg)</td>
<td>β-TBOH (µg/kg)</td>
<td>α-TBOH (µg [β-TBOH]/TBA equivalent per kg)</td>
<td>Total bound</td>
<td>Amount bioavailable</td>
</tr>
<tr>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>V</td>
<td>VI</td>
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<tr>
<td>Muscle (300)</td>
<td>3.2</td>
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<td>0.72</td>
<td>0.02</td>
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<tr>
<td>Liver (100)</td>
<td>50</td>
<td>0</td>
<td>1.4</td>
<td>0.35</td>
<td>4.8</td>
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<tr>
<td>Kidney (50)</td>
<td>22</td>
<td>0</td>
<td>0.71</td>
<td>0.06</td>
<td>2.1</td>
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<tr>
<td>Fat (50)</td>
<td>2.5</td>
<td>0</td>
<td>1.1</td>
<td>0.02</td>
<td>0.14</td>
</tr>
</tbody>
</table>

**Key to columns**

1. See section 2.6 of the main report.
2, 3, 4. Data obtained from residue studies.
5. Values given represent 10% of actual α-TBOH values. This converts α-TBOH to equivalent values for β-TBOH, on the assumption that the toxicological potency of α-TBOH is only 10% of that of β-TBOH.
6. For muscle, the value given is total residue (column II) less the sum of the free residues expressed as β-TBOH (columns IV + V). For liver, kidney and fat, the value given is calculated in the same way and then multiplied by 0.1. The assumption is that the bound residue in these three tissues is all in the form of α-TBOH and must be converted to β-TBOH equivalent.
7. Calculated by multiplying the values in column VI by 0.15, on the assumption that a maximum of 15% of the total bound residues is bioavailable.
8. Calculated by adding the values in columns IV, V and VII.
9. Calculated by multiplying the gram value in column I (expressed as kg) by the value in column VIII; for example, for muscle: 0.3 (kg) × 1.1 (column VIII) = 0.33.
### Annex 4

#### RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

<table>
<thead>
<tr>
<th>Substance</th>
<th>Acceptable Daily Intake (ADI) for human beings</th>
<th>Recommended Maximum Residue Level (MRL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthelmintic drug</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albendazole</td>
<td>0-0.05 mg per kg of body weight</td>
<td>Muscle, fat and milk: 0.1 mg/kg; Liver and kidney: 5 mg/kg</td>
</tr>
<tr>
<td>Antiprotozoal drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimetridiazole</td>
<td>Not allocated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ipronidazole</td>
<td>Not allocated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Not evaluated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ronidazole</td>
<td>0-0.025 mg per kg of body weight&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Antimicrobial sulfonamides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfadimidine&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0-0.004 mg per kg of body weight&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Meat, liver, kidney and fat: 0.3 mg/kg as total residue; 0.1 mg/kg as sulfadimidine; Milk: 0.05 mg/kg as total residue; 0.025 mg/kg as sulfadimidine</td>
</tr>
<tr>
<td>Sulfathiazole</td>
<td>Not allocated&lt;sup&gt;a&lt;/sup&gt;</td>
<td>No MRLs allocated&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Growth promoter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trenbolone acetate</td>
<td>0-0.02 µg per kg of body weight</td>
<td>Muscle: 2 µg/kg as β-trenbolone; Liver: 10 µg/kg as α-trenbolone</td>
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<tr>
<td>Trypanocides</td>
<td></td>
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<tr>
<td>Diminazene</td>
<td>Not allocated&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Isometamidium</td>
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<td>No MRLs allocated&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> Insufficient toxicological information was available to establish an ADI (see Annex 5).

<sup>b</sup> MRLs were not established because: (1) an ADI was not allocated; and (2) insufficient information was available (see Annex 5).

<sup>c</sup> This substance was not evaluated because toxicological data were not made available to the Committee.

<sup>d</sup> Temporary acceptance (see Annex 5).

<sup>e</sup> Insufficient information was available to establish MRLs (see Annex 5).

<sup>f</sup> This compound was on the agenda under the name "sulfamethazine", but was evaluated under the name "sulfadimidine", which is the international nonproprietary name for it.

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Annex 5

FURTHER TOXICOLOGICAL STUDIES AND OTHER INFORMATION REQUIRED OR DESIRED

Anthelmintic drug

Albendazole

A marker residue substance in milk needs to be identified and further information is required on the quantitative relationship between the concentration of 2-aminobenzimidazole sulfone and total residues in muscle, kidney and fat of cattle and sheep.

Antiprotozoal drugs

Dimetridazole

Before reviewing the compound again, the Committee would wish to see results from the following studies:

1. A long-term study in mice.
2. Studies aimed at investigating the mechanism of tumorigenesis.
3. Adequate studies of total residue depletion in poultry and swine using ring-labelled $^{14}$Cdimetridazole.
4. Metabolism studies in swine and poultry that characterize the total/bound residues.

Ipronidazole

Before reviewing the compound again, the Committee would wish to see results from the following studies:

1. Adequate in vitro and in vivo genotoxicity studies in mammalian systems.
2. A carcinogenicity study in rats to assess the effect of ipronidazole on the mammary gland and other tissues.
3. Studies aimed at investigating mechanisms that might explain the effect of the drug in increasing the incidence of mammary tumours in rats and lung tumours in mice.
4. An in vivo metabolism study in rats using ring-labelled ipronidazole. Special emphasis should be placed on the detection of metabolites in which the 5-nitro group has been reduced.
5. Adequate studies of total residue depletion in swine and turkeys using ring-labelled [$^{14}$C]pronidazole.
6. Metabolism studies in swine and turkeys that characterize the total residues.

**Metronidazole**

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

1. Comprehensive information on its toxicology so that a safety evaluation can be made.
2. The results of studies of total residue depletion in food-producing animals using ring-labelled [$^{14}$C]metronidazole.
3. The results of metabolism studies in food-producing animals that elucidate the nature of the total residue.

In addition, an analytical procedure needs to be developed for identifying and measuring the residues of metronidazole in the edible tissues of food-producing animals.

**Ronidazole**

The Committee requires the following by 1993:

1. A complete submission, including data on individual animals, of the carcinogenicity study reports.
2. The results of studies aimed at investigating the mechanism of tumorigenesis.

Before the Committee can establish MRLs, data establishing a relationship between a marker compound and the total residue will be needed. In addition, the toxicological significance of the bound residue will have to be further assessed.

**Antimicrobial sulfonamides**

**Sulfadimidine**

Results of studies known to be in progress on the effects of sulfadimidine on the thyroid gland in various animal species are required by 1991.
Sulfathiazole

Before reviewing the compound again, the Committee would wish to see results from the following studies:

1. Studies designed to assess the effects of sulfathiazole on sensitive parameters of thyroid and pituitary function in rodents.
2. Mammalian genotoxicity studies.
3. Studies designed to elucidate the metabolism of sulfathiazole.
4. Studies designed to identify and quantify the residues of sulfathiazole in food-producing animals by administration of $^{14}$C-labelled sulfathiazole.

Trypanocides

Diminazene

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

1. The results of a teratogenicity study.
2. The results of in vitro and in vivo mutagenicity studies so that the genotoxicity and carcinogenic potential of the compound can be evaluated.
3. The results of studies on residue levels in food-producing animals.
4. Information on available methods for measuring milk and tissue residues of diminazene.

Isometamidium

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

1. The results of studies designed to indicate the nature of the metabolites of isometamidium in the target species, and to provide an indication of the extent to which they are absorbed in laboratory species after oral administration.
2. The results of adequate mutagenicity studies so that the genotoxicity and carcinogenic potential of the compound can be assessed.
3. The results of a teratogenicity study with oral administration of isometamidium.
4. The results of a 90-day study in rats.
5. The results of studies on residue levels in food-producing animals.
6. Information on available methods for measuring milk and tissue residues of isometamidium.
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<td>(1987) WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
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<td>Prevention and control of intestinal parasitic infections</td>
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<td>Rheumatic fever and rheumatic heart disease</td>
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<td>Strengthening ministries of health for primary health care</td>
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</table>
767 (1988) Urban vector and pest control
Eleventh report of the WHO Expert Committee on Vector Biology and
Control (77 pages) .................................................. 9.—
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Health (71 pages) .................................................. 9.—
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787 (1989) WHO Expert Committee on Drug Dependence
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