EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

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

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Contents

1. Introduction ........................................................................................................ 1

2. General considerations ....................................................................................... 2
   2.1 Assessment of carcinogenic risk ................................................................. 2
   2.2 Quality of data ............................................................................................ 2
   2.3 Considerations on marker residues ........................................................... 3
   2.4 Response to the Codex Committee on Residues of Veterinary Drugs in Foods ................................................................. 4
   2.5 Project to update principles and methods for the risk assessment of chemicals in food ................................................................. 4

3. Comments on residues of specific veterinary drugs ........................................... 5
   3.1 Antimicrobial agents .................................................................................. 5
      3.1.1 Neomycin .......................................................................................... 5
      3.1.2 Flumequine ....................................................................................... 11
   3.2 Antiprotozoal agent ................................................................................... 16
      3.2.1 Imidocarb .......................................................................................... 16
   3.3 Insecticides ................................................................................................. 19
      3.3.1 Deltamethrin ..................................................................................... 19
      3.3.2 Dicyclanil ........................................................................................ 21
      3.3.3 Trichlorfon (metrifonate) ................................................................ 27
   3.4 Production aid ............................................................................................. 34
      3.4.1 Carbadox ......................................................................................... 34

4. Recommendations ............................................................................................... 41

Acknowledgement .................................................................................................. 41

References ............................................................................................................... 42

Annex 1
Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives ......................................................... 43

Annex 2
Recommendations on compounds on the agenda .................................................. 53

Corrigenda ............................................................................................................... 57
Joint FAO/WHO Expert Committee on Food Additives  
Geneva, 4–13 June 2002

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

*Toxicological evaluation of certain veterinary drugs in food*. WHO Food Additives Series No. 51, 2003

Residues monographs are issued separately by FAO under the title:

*Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/15, 2003

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**INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY**

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

The IPCS is a joint venture of the United Nations Environment Programme, the International Labour Organisation, 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.
Introduction

A meeting of the Joint FAO/WHO Expert Committee on Food Additives was held at World Health Organization (WHO) Headquarters, Geneva, from 6 to 12 February 2003. The meeting was opened by Dr David Nabarro, Executive Director, Sustainable Development and Healthy Environments, WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations (FAO) and WHO. Dr Nabarro recognized the recent retirement of Dr John Herrman and his contributions to JECFA over the past 18 years.

Dr Nabarro noted that the Committee would be responding to a request from the Codex Committee on Residues of Veterinary Drugs in Foods to define more clearly its policy with respect to risk assessment when establishing acceptable daily intakes (ADI)s and recommending maximum residue limits (MRLs) for veterinary drugs. He also discussed the current review of the Codex Alimentarius and the ratification in that review of the continuing need of Codex and Member States for independent scientific advice.

Fourteen meetings of the Committee had been held to consider veterinary drug residues in food (Annex 1, references 80, 85, 91, 97, 104, 110, 113, 119, 125, 128, 134, 140, 146 and 157) in response to the recommendations of a Joint FAO/WHO Expert Consultation held in 1984 (/). The present meeting was convened in response to a recommendation made at the fifty-fourth meeting of the Committee that meetings on this subject should be held regularly (Annex 1, reference 146). 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:

— to elaborate further principles for evaluating the safety of residues of veterinary drugs in food, for establishing ADIs and for recommending MRLs for such residues when the drugs under consideration are administered to food-producing animals in accordance with good practice in the use of veterinary drugs (see section 2); and
— to evaluate the safety of residues of certain veterinary drugs (see section 3 and Annex 2).

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1 As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives held in 1955 (FAO Nutrition Meeting Report Series, No. 11, 1956; WHO Technical Report Series, No. 107, 1956), there have been 59 previous meetings of the Joint FAO/WHO Expert Committee on Food Additives (Annex 1).
2. General considerations

2.1 Assessment of carcinogenic risk

The Committee was aware that both JECFA (Food Additives) and the Joint Meeting on Pesticide Residues (JMPR) had reviewed the IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis (7) and had adopted it as part of their working practices. The present Committee reviewed the approach proposed and agreed to use it for evaluating compounds for carcinogenic potential. The Committee further agreed to conduct a stepwise consideration of the risk posed by drugs that are carcinogenic to experimental animals, by answering the following questions:

- Did the compound produce tumours in experimental animals?
- Could a mode of action for formation of these tumours (each type being considered separately) be established?
- Was the mode of action such that a threshold might be anticipated?
- If so, had a clear threshold been demonstrated in the studies available?

The Committee noted that, even if a carcinogen gave positive results in some assays for genotoxicity, that was not necessarily the mode of action for tumour formation. The Committee further noted that there could be a threshold for some genotoxic mechanisms, particularly those in which damage to DNA is indirect, such as inhibition of spindle formation leading to aneugeny. In such cases, the Committee would evaluate risk in a similar way to other end-points for which there is a threshold.

The Committee agreed that, when it was not possible to conclude that a carcinogenic response was due to a mechanism that had a threshold, it would also not be possible to identify a dose that posed no risk. The Committee was not confident that any of the methods proposed to date for extrapolating to low doses was sufficiently reliable for the purpose of regulating residues of veterinary drugs in food. The Committee emphasized the need to consider compounds case by case.

2.2 Quality of data

The Committee has routinely addressed the quality of studies provided for its safety assessments of veterinary drugs. The Committee considered it appropriate to further elaborate its procedures for analysing the quality of submitted data and their use in assessments.

Most of the requirements for general toxicological data on food additives and contaminants are outlined in Environmental Health Criteria No. 70
and many of those requirements are applicable to veterinary drugs. The Committee at its forty-second meeting (Annex 1, reference 110) indicated the need for detailed reports that included data on individual animals from short-term studies of toxicity, long-term studies of toxicity and carcinogenicity and studies of reproductive and developmental toxicity and the results of assays for genotoxicity.

Similarly, the Committee at its twenty-sixth and twenty-seventh meetings (Annex 1, references 59 and 62) noted the general requirements for residue data, including comprehensive information on kinds and amounts of residues. Requirements with respect to analytical methods were noted in the report of the thirty-second meeting of the Committee (Annex 1, reference 80), and more specific requirements on the information to be requested were formulated in the reports of the forty-second and forty-seventh meetings (Annex 1, references 110 and 125). In addition, the Committee at its fifty-second meeting (Annex 1, reference 159) approved a document on requirements for analytical method validation, which provides guidance on the submission of analytical chemistry data, including the fitness of purpose for methods used to generate data on pharmacokinetics and residues and of a method for use in regulatory programmes.

Data requirements and guidance for writing expert reports on compounds with a long history of use were described in the report of the fortieth meeting of the Committee (Annex 1, reference 104), which stressed the importance of a comprehensive expert evaluation of the scientific literature and other relevant studies.

When the Committee is unable to establish clearly that adequate measures were in place to ensure that the conduct of the studies, the results and the analysis were consistent with recognized quality standards or protocols, it might decide not to consider the studies. The reasons for deciding not to consider a particular study are included in the Committee’s report. The Committee does not make unjustified or selective use of data or studies to expedite its evaluations, particularly when the data are inconsistent and the inconsistencies cannot be reasonably explained. Individual data or sets of data are not rejected unless adequate reasons for doing so can be stated.

2.3 Considerations on marker residues

The Committee at its fortieth meeting (Annex 1, reference 104) noted that the term “marker residue” was used in various ways in national programmes. The Committee applies the definition adopted by the Codex Committee on Veterinary Drugs in Foods. A marker residue is that residue the concentration of which decreases in a known relationship to the concentra-
tion of total residues in tissues, eggs, milk or other animal tissues. This definition applies to residues of toxicological and microbiological concern. Having a marker residue is important because it is used for determining compliance with MRLs and for related enforcement purposes by national governments.

Although the Committee has not explicitly stated its policy, the definition it uses is based on the principle that in virtually all instances the marker residue is a single (specific) compound. An exception would be stereoisomers (compounds of the same general chemical structure but differing in geometrical configuration at a single location in the molecule). Adherence to a single compound as a marker residue has several advantages for national authorities, in addition to simplifying the Committee’s recommendations on MRLs. A single analytical method is preferred for residue control purposes, it allows more monitoring and surveillance of residues in food animals, and, in general, it reduces the analytical uncertainties associated with residue analysis when compared with those situations in which more than one analysis may be required to determine compliance with an MRL. The Committee therefore affirmed the concept of selecting a single compound, whenever possible, as a marker residue and describing MRLs accordingly as residue equivalents of the parent veterinary drug.

2.4 Response to the Codex Committee on Residues of Veterinary Drugs in Foods

The Committee prepared a response to the request from the Codex Committee on Residues of Veterinary Drugs in Foods to define more clearly its policies with respect to risk assessment when establishing ADIs and recommending MRLs for veterinary drugs. The response will be transmitted to the Codex Committee and posted on the Expert Committee’s FAO and WHO websites.

2.5 Project to update principles and methods of risk assessment of chemicals in food

The Committee was informed of progress on the Joint FAO/WHO project to update the principles and methods for the risk assessment of chemicals in food. The Committee recognized the importance of this project and noted the issues to be covered, including evaluation of analytical and toxicological methods, intake assessment, dose–response modelling and harmonization of procedures for establishing MRLs. Those had all been important
considerations at the current meeting. The Committee was informed that drafts of the working papers would be available on the FAO and WHO websites for review, and comments were encouraged. The Committee strongly endorsed the effort and urged its continuing support.

3. Comments on residues of specific veterinary drugs

The Committee reconsidered two antimicrobial agents, one antiparasitic agent, three insecticides and one production aid. The recommendations made with regard to these substances and details of further studies and other information required are summarized in Annex 2.

Addenda to toxicological monographs were prepared on neomycin, flumequine, trichlorfon (metrifonate) and carbadox, and residues monographs were prepared on all the compounds considered in this section with the exception of trichlorfon.

3.1 Antimicrobial agents

3.1.1 Neomycin

Neomycin is an aminoglycoside antibiotic with a long history of use. It is indicated in the treatment of intestinal and respiratory infections, wound and skin infections and mastitis. It is available as neomycin sulfate and is formulated either alone or in combination with other antibiotics, such as lincomycin, penicillin, cephalosporins and some sulfonamides, for administration orally (including in feed and medicated drinking-water), by injection, by intramuscular infusion and by topical (including ocular) application. It is used parenterally at doses of 7–15 mg/kg bw in cattle, 7 mg/kg bw in sheep, 7–10 mg/kg bw in pigs and 7–21 mg/kg bw in poultry, expressed as neomycin base. The duration of treatment with neomycin sulfate is 3–10 days for poultry and up to 14 days for larger animals. Neomycin base is also administered as an intramammary infusion at 100–500 mg per mammary quarter at 12-h intervals for three successive milkings in dairy cattle and as a single dose for non-lactating dairy cattle after the last milking in a lactation cycle.

Toxicological data

The Committee considered neomycin at its forty-third, forty-seventh, fifty-second and fifty-eighth meetings (Annex 1, references J3, J25, J40 and J57). At its forty-third meeting, the Committee established a temporary
ADI of 0–30 µg/kg bw on the basis of a NOEL of 6 mg/kg bw per day for ototoxicity in a 90-day study in guinea-pigs and a safety factor of 200. The ADI was made temporary in view of deficiencies in the genotoxicity data. Studies of mutagenicity were requested for evaluation in 1996.

At its forty-seventh meeting (Annex 1, reference /25), the Committee considered new data on genotoxicity for neomycin. On concluding that neomycin was not genotoxic, it established an ADI of 0–60 µg/kg bw on the basis of the NOEL of 6 mg/kg bw per day for ototoxicity in the 90-day study in guinea-pigs and a safety factor of 100.

Following a request by the Codex Committee on Residues of Veterinary Drugs in Foods at its Twelfth Session (4), the Committee at its fifty-eighth meeting considered information on registration of injectable neomycin products and on their use with respect to good practice in the use of veterinary drugs. The Committee also considered data on the toxicity of neomycin in calves, but it concluded that the information was relevant only to the welfare of the target animals and therefore fell outside its mandate.

The Codex Committee on Residues of Veterinary Drugs in Foods at its Thirteenth Session (5) requested the Committee to evaluate new data on the safety of neomycin. Two submissions were made, one addressing the microbiological aspects of the safety of neomycin to consumers and the other addressing the evidence for a link between the presence of a specific mutation to mitochondrial DNA in humans and increased susceptibility to aminoglycoside-induced ototoxicity.

The ADIs that could be derived from studies in bacteria in vitro, from studies of human flora-associated animals in vivo and from studies in humans were evaluated. The available relevant microbiological data indicated that the toxicological ADI of 0–60 µg/kg bw would protect the gastrointestinal flora of humans. The lowest ADI that could be set on the basis of the results of all the available microbiological studies would be 0–14 mg/kg bw (840 mg per person). This value is higher than the existing ADI of 0–60 µg/kg bw (3.6 mg per person), which was set on the basis of toxicological data. Intake of residues at levels that would expose consumers to up to 60 mg/kg bw (3.6 mg per person) would not be expected to have adverse effects on the gut microflora. Consequently, there is no need to change the current ADI on the basis of the microbiological data.

The Committee affirmed that recent papers indicate that there is a causal relationship between the presence of a point mutation in which the adenine at position 1555 of the 12S rRNA gene on mitochondrial DNA is changed to a guanine (A1555G mutation) and the development of deafness. Hearing
loss can be due to both genetic and environmental factors. Systemic exposure to a large dose of aminoglycosides can bring about deafness, and genetic factors may make some people more susceptible to the ototoxic effects of aminoglycosides than others. Although people with the A1555G mutation appeared to be more susceptible to aminoglycoside-induced ototoxicity, it was not clear whether any of them had received neomycin. Nevertheless, it was considered prudent to assume that the effects observed were relevant to all aminoglycosides, including neomycin.

Some families with the A1555G mutation appeared to be at increased risk of hearing loss even in the apparent absence of exposure to aminoglycosides. Thus, people with this mutation may be more susceptible to ototoxicity caused by a variety of environmental factors, one of which is exposure to aminoglycosides. The mutation is inherited from the mother and has been demonstrated in various ethnic groups, including Chinese, Japanese, Mongolian, Spanish and Arab–Israeli, and in individuals in the USA of diverse origin. Only a few families and individuals with the A1555G mutation have been identified worldwide.

The Committee recognized that people with the A1555G mutation might be susceptible to aminoglycoside-induced ototoxicity and might become deaf after receiving therapeutic doses of aminoglycosides. Since the dose and route of administration of aminoglycosides were not given in the reports of the studies in humans, a dose–response relationship could not be established for an increased risk of ototoxicity after administration of aminoglycosides to people with the A1555G mutation. No quantitative data were available for identifying a NOEL for the ototoxicity of neomycin or any other aminoglycoside in people with the A1555G mutation.

It was noted that the current ADI for neomycin of 0–60 μg/kg bw was set by applying a safety factor of 100 to the NOEL of 6 mg/kg bw per day for ototoxicity in a 90-day study in guinea-pigs. This safety factor comprises a 10-fold factor to compensate for extrapolation of results from guinea-pigs to humans and another 10-fold factor to account for interindividual variation within the human population.

The Committee was aware that systemic exposure to large doses of aminoglycosides in excess of the recommended therapeutic doses could result in deafness in any person, irrespective of the presence of the mitochondrial DNA mutation. Nevertheless, deafness has been reported in people with the A1555G mutation given therapeutic doses of aminoglycosides. The recommended oral therapeutic dose of neomycin for adults is about 12 000 mg per person per day. The Committee noted that this dose is more than 3000 times greater than the current ADI for neomycin of
0–60 μg/kg bw (3.6 mg per person). This ADI is adequate to assure the health of all consumers, including those with the A1555G mutation.

The Committee concluded that there was no need to alter the ADI of neomycin to account for the possible susceptibility of the subpopulation with the A1555G mutation or to account for the microbiological properties of neomycin.

**Residue data**

At its forty-third meeting, the Committee recommended temporary MRLs which included 5000 μg/kg for kidney, 500 μg/kg for liver and 500 μg/kg for cows’ milk. The Committee at its forty-seventh meeting increased the MRL for kidney to 10 000 μg/kg and proposed that all the other temporary MRLs recommended by the Committee at its forty-third meeting be adopted. In response to a request from the Codex Committee on Residues of Veterinary Drugs in Foods at its Thirtieth Session to evaluate new data on safety, the Committee at its fifty-second meeting increased the MRLs for cattle liver and kidney to 15 000 μg/kg and 20 000 μg/kg, respectively, in order to accommodate use of injectable formulations.

The Committee at its fifty-eighth meeting received data to support an increase in the MRL for milk and reconsideration of the MRLs for cattle liver and kidney. In the light of a request from the Codex Committee on the Residues of Veterinary Drugs in Foods at its Thirteenth Session to evaluate new data on safety, however, the Committee at its fifty-eighth meeting recommended that the MRLs that it had recommended at its forty-seventh and fifty-second meetings be maintained and that a review of the MRLs be deferred until such time as the toxicology of neomycin was re-evaluated.

The Committee at its present meeting considered data that had been submitted to support an increase in the MRL for milk and reconsideration of the MRLs for cattle liver and kidney.

Two studies on depletion of neomycin residues, which complied with good laboratory practice, one in milk and the other in kidneys and liver of cattle, were evaluated. An additional study of tissue residues in heifers, which did not comply with good laboratory practice, was not taken into account as the animals received less than half the total dose administered in the first study and the dose was lower than that currently recommended.

The study on the depletion of neomycin residues in milk was conducted in healthy lactating dairy cows which received intramammary infusions of a combination of 100 mg neomycin base and 330 mg lincomycin base into each mammary quarter at 12-h intervals after three successive milkings.
Pooled (composite) milk samples were collected from all cows before each infusion until the tenth milking after the last infusion. Milk samples were assayed for neomycin by solid-phase extraction and high-performance liquid chromatography (HPLC) with a limit of quantification of 0.1 μg/ml. Statistical tolerance limits were calculated for the concentration of residue versus depletion over time. Upper limits of 1800 μg/kg, 1500 μg/kg and 1000 μg/kg were determined for neomycin concentrations in milk samples at 72 h, 76 h and 84 h, respectively.

The study of depletion of neomycin in kidneys was conducted in non-ruminating bull calves weighing about 35 kg. The animals were given neomycin sulfate orally at a dose equivalent to 15 mg/kg bw as neomycin base for 14 consecutive days. Liver and kidney samples were collected 7, 14, 21 and 28 days after treatment. The concentrations of neomycin in kidney and liver were measured by a microbiological method. The limit of quantification was 0.92 μg/g in liver but was not reported for kidney. The study was initially designed for determination of residues in kidneys only; however, liver samples collected at the same time were analysed for neomycin residues 16 months later, although these data were not supported by data on stability in storage. The statistical tolerance for kidney was greater than 10 000 μg/kg at all times. The concentrations in liver were below the limit of quantification from day 14 through day 28, except for individual animals on days 21 and 28, in which concentrations of 2200 μg/kg and 1200 μg/kg were found, respectively. The few data for both kidney and liver were inadequate for interpretation.

**Effect on starter cultures**

The effect of neomycin on bacterial starter cultures used in the production of Italian cheese, yoghurt, buttermilk and sour cream was assessed from time-to-clot ratios. Concentrations in milk up to 2 mg/l were shown to have no effect on the growth of bacteria in any of the starter cultures. Studies on the effect of neomycin residues on pH during fermentation were not reported.

**Analytical methods**

Data were provided for validation of a HPLC method for the determination of neomycin residues in cows’ milk, while the concentrations in kidneys and liver were determined by a microbiological method.

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1 Statistical tolerance limits for the residue concentration–time depletion curve were determined by calculating the linear regression of the logarithmic concentrations of neomycin in milk versus time and then calculating the upper one-sided 95% confidence interval for the 95th percentile of the population.
Milk samples from untreated animals were fortified with neomycin at concentrations of 50–5000 μg/ml. After clean-up of the samples by solid-phase extraction, the residue was analysed by HPLC with post-column derivatization and fluorescence detection. The average amount of neomycin recovered from 15 fortified milk samples was 59%. The precision, measured as intra-day and inter-day repeatability, and the linearity of the calibration curves were acceptable for all concentrations of neomycin in cows’ milk, with a limit of quantification of 0.10 μg/ml. The specificity for differentiation from matrix components and from lincomycin was assessed, and no interference was found in either situation. Neomycin in milk stored at temperatures of −20 °C or less was stable up to 210 days.

*Maximum residue limits*

The Committee considered the following factors in recommending MRLs:

- An ADI of 0–60 μg/kg bw based on a toxicological end-point has been established, which would result in a maximum daily intake of 3600 μg for a 60-kg person.
- Aminoglycosides undergo negligible metabolism after parenteral administration to animals, and the parent drug represents all the residues present.
- Neomycin is the marker residue for tissues, milk and eggs.
- A validated HPLC method with a limit of quantification of 0.10 μg/ml for neomycin in cows’ milk is available that could be used routinely in many laboratories.
- Concentrations of neomycin up to 2 mg/kg had no effect on time-to-clot ratios of bacterial starter cultures used in the production of fermented milk products.
- Data on residues in milk supported an MRL for cows’ milk of 1500 μg/kg.
- Information on the registered use patterns for injectable formulations of neomycin in food-producing animals was requested from governments and considered. The information indicated that use of parenteral formulations is not regarded as good practice in the use of veterinary drugs, and few such products were found to be approved.
- The MRLs for kidney of 20 000 μg/kg and for liver of 15 000 μg/kg recommended by the Committee at its fifty-second meeting to accommodate use of parenteral formulations are therefore unnecessary.

The Committee, having considered the database submitted since its forty-third meeting, decided to revert to the MRLs for cattle kidney and liver that it had recommended at its forty-seventh meeting.
On the basis of the above considerations, the Committee recommended the following MRLs: cattle kidney, 10 000 μg/kg; cattle liver, 500 μg/kg; and cows' milk, 1500 μg/kg. The MRLs of 500 μg/kg for cattle muscle and fat were maintained.

Assuming consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat, 1.5 kg of milk and 100 g of eggs, the theoretical maximum daily intake of neomycin residues would be 3000 μg. This accounts for 83% of the ADI of 3600 μg for a person weighing 60 kg.

3.1.2 Flumequine

Flumequine is a fluoroquinolone compound with antimicrobial activity against gram-negative organisms and is used in the treatment of enteric infections in food animals. It also has limited use in humans for the treatment of urinary-tract infections. Flumequine was evaluated by the Committee at its forty-second, forty-eighth and the fifty-fourth meetings (Annex 1, references 110, 128 and 146). At its forty-eighth meeting, the Committee established an ADI of 0–30 μg/kg bw based on a toxicological end-point (hepatotoxicity in male CD-1 mice in a 13-week study), and recommended MRLs for flumequine of 500 μg/kg for muscle and liver, 3000 μg/kg for kidney and 1000 μg/kg for fat in cattle, pigs, sheep and chickens, expressed as parent drug. The Committee also recommended an MRL of 500 μg/kg for trout muscle with skin in normal proportions.

At its forty-eighth meeting, the Committee evaluated information related inter alia to a NOEL for hepatotoxicity and the mechanism of tumour induction. The present Committee, at the request of the Codex Committee on Residues of Veterinary Drugs in Foods at its Thirteenth Session (5), evaluated new studies that had been carried out to elucidate further the mechanism of flumequine-induced hepatocarcinogenicity in mice.

Toxicological data

In short-term and long-term studies of toxicity that had been evaluated by the Committee at its forty-second meeting, oral administration of flumequine caused dose-related hepatotoxic effects in rats and mice. Hypertrophy, degenerative changes and focal necrosis of hepatocytes were observed in rats at 400 and 800 mg/kg bw per day in a 2-year study, and in CD-1/ICR mice at 400 and 800 mg/kg bw per day in an 18-month study. The prevalence of hepatotoxic lesions increased with duration of treatment. At its forty-eighth meeting, the Committee noted that male mice were the most sensitive to flumequine-induced liver damage.
The results of long-term studies that had been evaluated previously by the Committee showed no carcinogenic effects in rats, but a dose-related increase in the incidence of liver tumours was observed in CD-1 mice at doses greater than 100 mg/kg bw per day. The tumour incidence paralleled hepatotoxic changes and was significantly higher in male than in female mice. As flumequine was inactive in a range of tests for genotoxicity, including assays for gene mutation in bacteria and mammalian cells in vitro and for chromosomal aberrations in vivo and in vitro, the mechanism of tumorigenicity was unclear. At its forty-eighth meeting, the Committee reviewed the available toxicological database in order to determine if the hepatocarcinogenicity of flumequine resulted from a genotoxic or a non-genotoxic mechanism. Genotoxic carcinogens act directly on DNA in the target tissue, inducing DNA or chromosomal damage such as strand breaks or mutations, which can typically be assessed in assays for genotoxicity and short-term assays in rodents. Non-genotoxic carcinogens do not have this activity; non-genotoxic carcinogenicity can result from induction of cytotoxicity and cell proliferation, which probably cause tumorigenicity in target organs by sustained mitogenic stimulation. Non-genotoxic tumorigenesis in rodent liver can arise through several mechanisms, including hepatotoxicity. Flumequine produced consistently negative results when evaluated in various assays for genotoxicity in vitro and in vivo.

Flumequine was hepatotoxic, causing hepatocellular degeneration and focal necrosis in male and female mice. The dose-related severity of the lesions paralleled the incidence of liver tumours. The occurrence of foci of altered hepatocytes is an important intermediary step in hepatotoxicity-induced liver tumorigenicity.

The Committee at its forty-eighth meeting concluded that the liver tumorigenicity observed in mice exposed to flumequine was the result of a non-genotoxic mechanism, secondary to hepatotoxicity-induced necrosis–regeneration cycles. The Committee noted that, as the tumorigenicity was secondary to hepatotoxicity, the NOEL for both hepatotoxicity and carcinogenicity was 25 mg/kg bw per day. The Committee also noted that the NOEL for hepatotoxicity was determined from a 13-week study and extrapolated to the dose required for tumour formation observed at the end of the 18-month study in mice.

The Committee at its present meeting evaluated new information on the mechanism of action of flumequine-induced mouse liver tumorigenicity. Administration of flumequine in the diet of CD-1 mice at a concentration of 4000 ppm for 30 weeks (equivalent to the lowest dose in the 18-month study of carcinogenicity in mice) or after a single intraperitoneal injection of N-nitrosodimethylamine induced basophilic liver foci in males. Flumequine
also increased the number of 8-hydroxydeoxyguanosine adducts in liver. These responses are consistent with oxidative DNA damage and can be associated with carcinogenicity. In another study, heterozygous \( p53 \)-deficient mice (which have increased sensitivity to genotoxic carcinogens) that received a diet containing 4000 ppm of flumequine for 26 weeks developed basophilic liver foci at a time when there was no evidence of necrosis. The absence of cell death at the dose tested (which did not cause necrosis) showed that this would not confound interpretation of the significance of the altered liver foci. In a recent 13-week, two-stage study of hepatocarcinogenicity in mice, administration of a diet containing flumequine at a concentration of 4000 ppm induced altered liver foci in mice subsequently exposed to a mixed promoting regimen of D-galactosamine and phenobarbital, indicating that flumequine was a short-term initiator. A lower dose of flumequine (500 mg/kg bw) caused DNA strand breaks in a “comet” assay at a time when liver damage was not evident. The absence of liver damage at the dose tested showed that this would not confound interpretation of the comet assay. The liver was more sensitive if it was undergoing cell proliferation due to regeneration or juvenile growth. Similarly, other tissues such as stomach, colon and urinary bladder showed more DNA breaks in response to various doses of flumequine, with dose-dependent DNA damage in these organs in adult mice 3 h, but not 24 h, after treatment. The results of these studies suggest that flumequine has initiating potential and that the hepatocarcinogenicity in mice might involve DNA strand breakage.

Quinolones like flumequine exert their antibacterial activity by inhibiting bacterial topoisomerase II (DNA gyrase). Although there are some structural similarities between bacterial and mammalian topoisomerases, they differ substantially in overall structure. Fluoroquinolones in general have a much lower affinity for mammalian topoisomerases than for bacterial enzymes. Information on inhibition of mammalian topoisomerases by flumequine was not available to the Committee at its forty-eighth meeting.

Flumequine at doses that inhibit mammalian topoisomerase II might induce DNA damage, and the damage might be involved in mutagenic or other genotoxic steps in carcinogenicity. The Committee noted that there was inadequate evidence to confirm that the observed carcinogenicity of flumequine was secondary to inhibition of topoisomerase II or that this hypothesized mechanism is the sole basis for the tumour induction observed in the lifetime study of carcinogenicity in mice. The Committee at its forty-eighth meeting considered that hepatocellular necrosis and regeneration subsequent to hepatotoxicity was the relevant mechanism for the induction of liver tumours by flumequine.
The Committee at its forty-second and forty-eighth meetings evaluated the tumorigenicity of flumequine. At its forty-second meeting, the Committee noted that there was evidence of compound-related hepatotumorigenic effects in male mice. As flumequine was inactive in a range of tests for genotoxicity, the mechanism of tumorigenesis was unclear.

Flumequine has generally been considered to be a non-genotoxic carcinogen with only promoting activity. The Committee at its present meeting reviewed new studies on flumequine-induced tumorigenicity that were not available at the forty-eighth meeting, in which the mechanism of action in male mice was further investigated. Although the results of the comet assays indicated that flumequine caused double-strand DNA breaks, the Committee noted the limitations of this assay and considered that those results alone could not fully substantiate a genotoxic mechanism for the observed hepatocarcinogenicity of flumequine.

The Committee concluded that the new data raised further questions about the mechanism by which flumequine increases the incidence of liver tumours in male mice. The Committee evaluated evidence that supported the involvement of both genotoxic and non-genotoxic mechanisms. It noted that flumequine was not genotoxic in a comprehensive battery of assays in vitro and in vivo. However, in the absence of necrosis, it induced basophilic foci and DNA strand breaks in the comet assay. The Committee therefore could not dismiss the possibility that flumequine induces tumours in mouse liver by a mechanism that includes genotoxic effects. It was, however, unable to identify the genotoxic effects involved in liver tumour formation or a threshold for those effects.

The Committee therefore concluded that it could not support an ADI and withdrew the ADI that it had established at its forty-eighth meeting. Before establishment of an ADI can be considered, the Committee would wish to receive additional data on the genotoxic effects involved in tumour formation.

**Residue data**

The Committee reviewed a new study on the disposition and depletion of residues of flumequine in black tiger shrimp (*Penaeus monodon*) for the purposes of recommending MRLs in giant prawns and black tiger shrimp. No information regarding the compliance of this study with GLP was available. The study provided information on pharmacokinetics and residues. Groups of black tiger shrimp with an average weight of 20–30 g were given flumequine at a dose of 12 mg/kg bw by intramuscular injection, oral gavage or in pelleted feed given ad libitum for 5 consecutive days.
The dose of 12 mg/kg bw is that recommended for fish. The shrimp were maintained in 5 × 10 m concrete tanks with the water temperature maintained at 28–32 °C. The half-lives for depletion in muscle tissue after intramuscular and gavage administration were 33 h and 60 h, respectively. The area under the curve of the concentration in muscle over time after gavage was one-fifth of that after intramuscular administration.

In the studies of residues, groups of nine random samples were collected at 20 times between 1 h and 312 h after dosing. The peak concentration in shrimp muscle, 2600 μg/kg, was reached 2 h after intramuscular injection, and the flumequine concentration remained above 250 μg/kg for 24 h. The concentration decreased to below the limit of quantification (LOQ), 5 μg/kg, 216 h after administration. After gavage, the peak concentration in shrimp muscle, 360 μg/kg, was observed by 12 h and decreased to below the LOQ by 144 h after administration. After administration in feed, the concentrations of flumequine were low (30–46 μg/kg) and decreased to below the LOQ by 96 h after treatment. When medicated feed was given ad libitum, the flumequine concentrations in tiger shrimp were below 500 μg/kg in all samples analysed.

**Analytical methods**

The flumequine concentrations in muscle tissue were determined by high-performance liquid chromatography with fluorescence detection. Calibration curves in muscle tissue were established by analysing fortified samples representing the range 0.5–30 μg/kg and 100–2000 μg/kg (their specific application was not described). The reported LOQ of the method was 5 μg/kg, and the calibration curves were linear over the tested range. The recovery was reported to be 99.8% (2000 μg/kg) to 104.4% (5 μg/kg).

After considering the new data, the Committee concluded that, if an ADI had been retained, the data would justify establishing a MRL of 500 μg/kg in muscle of black tiger shrimp, provided the following information was made available:

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

In view of the recommendation to withdraw the ADI for flumequine, the Committee agreed to withdraw the MRLs that had been established at previous meetings, for all species.
3.2 Antiprotozoal agent

3.2.1 Imidocarb

Imidocarb is an anti/protozoal drug which has been used since the 1970s for the treatment of babesiosis in cattle and sheep and anaplasmosis in cattle. It was evaluated by the Committee at its fiftieth meeting (Annex 1, reference /3A/), when it established an ADI of 0–10 μg/kg bw and temporary MRLs for edible tissues of cattle and for cattle milk, expressed as parent drug, as follows: muscle, 300 μg/kg; liver, 2000 μg/kg; kidney, 1500 μg/kg; fat, 50 μg/kg; cows’ milk, 50 μg/kg. The Committee requested the following additional information for evaluation:

— the results of residue depletion studies in which lactating and non-lactating cattle are given the recommended dose of imidocarb dipropionate by subcutaneous injection, with analysis of samples by the proposed regulatory method with enzymic digestion; and
— the results of a residue depletion study in sheep, in which the recommended dose and route of administration of imidocarb are used.

The new data provided addressed the request for additional data on the depletion of residues in lactating and non-lactating cattle treated with imidocarb dipropionate at the recommended dose and for analysis of the samples by a method that includes enzymatic digestion to release bound residues. Additional data were provided to support modifications to the method reviewed by the Committee at its fiftieth meeting. No new data were provided on use in sheep.

Residue data

A study conducted according to good laboratory practice (GLP) of residue depletion in tissues of non-lactating cattle given imidocarb dipropionate at the recommended dose of 3.0 mg/kg bw by subcutaneous injection confirmed the pattern of distribution of residues in various tissues seen in the GLP study with [14C]imidocarb dipropionate which had been reviewed by the Committee at its fiftieth meeting. Differences in the ages and weights of the animals, treatment regimens, sampling times and analytical methods complicate direct comparison with the results of earlier studies with unlabelled material. In the recent study, in which assays including enzyme treatment to release bound residues were used, the highest concentrations of residues were found 30 days after dosing in kidney (14 mg/kg), followed by liver (4.1 mg/kg), muscle (0.64 mg/kg) and fat (0.09 mg/kg). The residues in tissues were persistent: 60 days after dosing, 4.6 mg/kg was present in kidney, 1.4 mg/kg in liver, 0.27 mg/kg in muscle and 0.03 mg/kg in fat.
Most samples of edible tissues contained detectable residues 180 days after treatment but at concentrations well below the temporary MRLs recommended by the Committee at its fiftieth meeting. Nevertheless, all kidney samples and one liver sample tested 90 days after treatment contained concentrations above these MRLs. The residues in kidney determined in the analysis with enzymatic digestion were higher than those reported with methods that did not include this step, on the basis of which the fiftieth Committee established a higher temporary MRL for liver than for kidney. In view of the new data, the present Committee considered that the MRLs it had recommended at its fiftieth meeting did not reflect the distribution of parent compound in kidney and liver.

The results of the GLP study of depletion of imidocarb residues in milk from dairy cattle that had received a single treatment at the recommended dose of 3.0 mg/kg bw were similar to those of the studies reviewed by the Committee at its fiftieth meeting. The concentration peaked at 0.93 mg/kg at the second milking after treatment, then declined rapidly to 0.11 mg/kg at the sixth milking and 0.05 mg/kg at the eighth milking. The continued slow elimination resulted in concentrations below the temporary MRL of 50 μg/kg in milk from all animals from the tenth milking onwards.

**Analytical methods**

A suitably validated method was submitted to support the proposed MRLs for the marker residue, imidocarb free base. The critical control points in the method, such as silylation of glassware and keeping solid-phase extraction cartridges wet during this sample processing step, were identified. The recovery and precision met the requirements for regulatory methods established by the Codex Alimentarius (6). Information on the specificity of the method and the stability of the analyte were provided. The method includes an internal standard, allowing correction for recovery, and data were provided to indicate that the method performs in a satisfactory manner at the temporary MRLs established by the Committee at its fiftieth meeting and at the MRLs recommended by the Committee at the present meeting. The method is suitable for use in a routine residue control laboratory.

**Maximum residue limits**

In recommending MRLs, the Committee considered the following:

— An ADI of 0–10 μg/kg bw was established by the Committee at its fiftieth meeting, which results in an ADI of 0–600 μg for a 60-kg person.
— The percentages of marker residue to total residues determined in the study with radiolabelled compound considered by the Committee at
its fiftieth meeting were as follows: liver, 68%; kidney, 88%; muscle, 88%; milk, 77%. As no data were available for fat, a factor based on the lowest ratio reported (in liver) was applied by the Committee at its fiftieth meeting. The new data considered by the Committee at its present meeting confirmed the concentrations predicted from the data for total residues in fat (reviewed by the Committee at its fiftieth meeting) obtained with this factor. The present Committee rounded the percentages and assigned factors for correction of marker-to-total residues as follows: liver, 0.7; kidney, 0.9; muscle, 0.9; fat, 0.7; milk, 0.8.

— The recommended MRLs are based on data resulting from treatment of cattle with the recommended therapeutic dose of 3.0 mg/kg bw administered as a single subcutaneous injection.

— Imidocarb free base is the appropriate marker residue, as determined by the Committee at its fiftieth meeting.

— The new data on residue depletion indicate that kidney and muscle are the recommended target tissues.

— A suitable analytical method is available for analysis of imidocarb free base residues in edible tissues of cattle and in cows’ milk.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, expressed as imidocarb free base: muscle, 300 μg/kg; liver, 1500 μg/kg; kidney, 2000 μg/kg; and fat, 50 μg/kg. The Committee also recommended an MRL of 50 μg/kg for imidocarb in cows’ milk.

These recommended MRLs would result in a daily maximum intake of 520 μg, representing 87% of the ADI, on the basis of a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5 kg of milk.

In the absence of the additional data that had been requested, the temporary MRLs for kidney, liver, muscle and fat of sheep recommended by the Committee at its fiftieth meeting were withdrawn.

Guidance to national authorities

The Committee was aware that imidocarb is used therapeutically in individual animals, whereas prophylactic use at the recommended dose of 1.2 mg/kg bw may involve groups of animals. The recommendations based on therapeutic use may thus require adjustment to reflect local practice, including prophylactic use.

Irrespective of the treatment regimen, residues of imidocarb are persistent in both tissues and milk of treated animals. Use of this drug in lactating
dairy cattle may thus pose a challenge for national regulatory authorities, who must balance considerations of animal welfare against issues of the environment and food security associated with prolonged periods of discard after treatment. The studies with treatment of dairy cattle at the therapeutic dose of 3.0 mg/kg bw indicated that a minimum discard period of 96 h (normally, eight milkings) is required for co-mingled milk from a herd, assuming that one-third of the animals have been treated. The Committee considered that the discard period could be reduced in such circumstances by 12 h if the MRL for milk was 100 μg/kg. This would increase the theoretical maximum daily intake (TMDI) to 620 μg, resulting in a theoretical daily consumption that exceeds the ADI of 600 μg by 3%. National authorities considering this option should refine the estimates of intake to reflect national consumption factors in order to determine whether the higher MRL would result in an intake that exceeds the ADI.

3.3 **Insecticides**

3.3.1 **Deltamethrin**

*Residue data*

Deltamethrin is a synthetic insecticide belonging to the pyrethroid class and is used in particular for control of flies and lice in veterinary use. For veterinary use, it is applied topically as a dip, spray or a pour-on preparation to cattle, sheep, pigs, poultry and salmon.

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluated deltamethrin toxicologically in 1980, 1981 and 1982 (7–9) and established an ADI of 0–10 μg/kg bw in 1982. MRLs were recommended for veterinary use in 1990 and for plant protection purposes in 1982 by the JMPR. MRLs in cattle, sheep and chicken muscle, liver, kidney, liver and fat, salmon muscle, cows’ milk and eggs were recommended by the Committee at its fifty-second meeting (Annex 1, reference 140). At that meeting, the Committee noted that the concentrations of residues in muscle, milk and eggs were less than twice the limit of quantification of the analytical methods used and, therefore, recommended MRLs based on the limit of quantification of the methods in cattle, sheep, chicken and salmon muscle, in cows’ milk and in eggs, all expressed as parent drug.

The Codex Committee on Residues of Veterinary Drugs in Foods at its Thirteenth Session (.5) commented on information relevant to the intake of deltamethrin from use as a pesticide and veterinary drug. In particular, it expressed concern about the MRLs recommended by the Committee at its fifty-second meeting and the possible amendment of the MRLs for
veterinary drug residues to accommodate information with regard to dietary intake from pesticide use and veterinary drug use.

The JMPR at its 2002 meeting evaluated the dietary risk of pesticide residues in foods, including those from agricultural products and food animals, using procedures developed in 1999. The long-term dietary intake of deltamethrin residues was estimated as the international estimated daily intake (IEDI). Dietary intakes were calculated by multiplying the concentrations of residue expressed as ‘supervised trial median residue values’ (STMRs) by the average daily per capita consumption estimated for each commodity on the basis of the WHO Global Environmental Monitoring System (GEMS) Food diets. Long-term dietary intakes were expressed as a percentage of the ADI for a 60-kg person. For calculations of dietary intake, JMPR considered that, for intake from mammalian sources, 20% of cattle meat contains residues at the MRL for fat and 80% contains residues at the MRL for meat with trimmable fat removed. For calculations of intake from poultry meat, JMPR used 10% and 90%, respectively, for fat and muscle tissue for both fat-soluble and non-fat-soluble pesticides.

For deltamethrin, with an ADI of 0–10 µg/kg bw, the long-term intake estimated by the JMPR was rounded to 20–30% of the ADI. For residue concentrations in cattle fat, the JMPR used 0.19 mg/kg as the high concentration and a STMR of 0.16 mg/kg. The STMR is the residue concentration selected from the pesticide residue study (conducted at the recommended dose) yielding the median of the highest concentration of residue for a particular commodity. The residue concentration in cattle muscle was 0.027 mg/kg, and the STMR was 0.01 mg/kg. The corresponding values in poultry fat were 0.09 mg/kg and 0.038 mg/kg. The values in poultry muscle were 0.02 mg/kg for the high residue concentration and 0.02 mg/kg for the STMR. As the Committee’s recommendations for MRLs were the same as or higher than those recommended by the JMPR and they covered the main commodities (meat, kidney and liver of cattle, goats, pigs and sheep and poultry) for which JMPR recommendations were made, JMPR decided to use the recommendations of the Committee at its fifty-second meeting for estimating dietary intake.

Intake of deltamethrin residues from all agricultural food sources, on the basis of the JMPR algorithm and the GEMS/Food database, did not exceed 25% of the ADI (about 150 µg). The percentage of the ADI attributed to the IEDI (long-term exposure) to residues from food-producing animals was 0–1%, depending on which of the five GEMS diets was considered.
The Committee at its fifty-second meeting took account of the evaluations of deltamethrin by the JMPR. The Committee at its present meeting estimated that the daily theoretical maximum intake of residues from veterinary drug use would be 250 μg. This estimate is based on a theoretical diet containing 300 g of muscle, 100 g of liver, 50 g of kidney and fat, 1.5 kg of milk and 100 g of eggs, and the worst-case situation in which the marker residue deltamethrin accounted for 4% of the total residues in liver, 3% in kidney and 60% in fat. The results of studies of residues in milk considered by the Committee at its fifty-second indicated a concentration of 10 μg/kg, or 15 μg in 1.5 kg of milk. The estimated upper limit of the theoretical maximum intake from veterinary use would be 265 μg. The theoretical intake of deltamethrin residues from pesticide use would represent 25% of the ADI, equivalent to 150 μg. The sum of the theoretical concentrations of deltamethrin residues resulting from use as a veterinary drug and pesticide would be no more than 415 μg, equivalent to 68% of the ADI in the most conservative estimate.

On this basis, the Committee affirmed that the MRLs it had recommended at its fifty-second meeting were compatible with the ADI and noted that the analysis reported here addressed the request of the Codex Committee on Residues of Veterinary Drugs in Foods at its Thirteenth Session.

3.3.2 Dicyclanil

Residue data

Dicyclanil (5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine) was first reviewed by the Committee at its fifty-fourth meeting (Annex 1, reference 146) for use as an insect growth regulator in sheep. MRLs for muscle, liver, kidney and fat tissues were set. The report of the Codex Committee for Residues of Veterinary Drugs in Foods at its Thirteenth Session (5) noted that several delegations had indicated "that use of dicyclanil itself as a marker residue gave an estimated total maximum daily intake far above the ADI" and therefore recommended that the Committee consider the issue at its sixtieth meeting in February 2003.

The present re-evaluation of dicyclanil residues was undertaken to address the question of the allocation of MRLs for various tissues, the appropriate marker residue and the suitable analytical procedure. In its previous review, the Committee suggested that dicyclanil should be used as the marker residue instead of the sum of dicyclanil and the metabolite 2,4,6-triaminopyridimidine-5-carbonitrile (CGA297107). Concern was expressed that the consequence would be that the theoretical maximum daily intake (TMDI)
would exceed the ADI. The Committee therefore decided to re-evaluate the MRLs and to estimate the effect of allocation of the marker residue on the suggested MRLs.

The Committee reviewed all the studies that had been conducted to determine the effects of factors such as breed, type and length of the wool, time after shearing and the applied dose on the concentrations of dicyclanil-related residues in edible tissues. Merino sheep were used in most of the studies, whereas statistical analysis of the results indicated that there were differences among the breeds in terms of residue kinetics. Different types of fat and muscle tissues from various parts of the body were pooled when appropriate. The Committee noted that there were inconsistencies between the metabolic profile established for radiolabelled dicyclanil and the residues found in subsequent residue depletion studies with non-radiolabelled compound. These inconsistencies were evaluated by the Committee and found to have no impact on the selection of recommended MRLs when the TMDI was estimated on the basis of the sum of dicyclanil and CGA297107.

The previous evaluation addressed studies conducted with [14C]dicyclanil administered orally to rats. The radioactivity was essentially completely recovered in urine and faeces. The most significant metabolic route was conversion of dicyclanil to N-(4,6-diamino-5-cyano-pyrimidin-2-yl)propionamide, which constituted 50% of the administered dose. The metabolite CGA297107 constituted 11% of the metabolites.

Six studies of administration of [14C]dicyclanil to sheep comprised three studies of the disposition of the substance after topical administration of 1.2 g (35 mg/kg bw), 1.5 g (35 mg/kg bw) and 100 mg/kg bw, and three in which tissue samples and excreta from these studies were used to characterize dicyclanil-related compounds. About 2–4% of the dose was absorbed during the first 168 h. Dicyclanil-related compounds were excreted in almost equal proportions in urine and faeces. Of the amount excreted, 59–80% represented parent dicyclanil and only 10% and 4.9% consisted of CGA297107 in the two first studies, respectively. Therefore, dicyclanil was not extensively metabolized in sheep.

The residues in the studies with radiolabelled compound were extracted with acetonitrile, methanol and hexane. In some studies, microwave and Soxhlet extraction was also used, and other metabolites tended to break down to the metabolite CGA297107 under these harsh conditions.

The third study with radiolabelled compound addressed the metabolite profile in tissues 7 and 21 days after administration. Dicyclanil comprised 13% and CGA297107 constituted 11% of the total residues in liver 7 days
after administration and 4.8% and 8.0% 21 days after administration, respectively. The concentrations of unextractable residues remained virtually constant over time. In kidney, the amount of dicyclanil-related radioactivity was low, and the extractable residues 7 days after administration consisted of 24% dicyclanil and 21% CGA297107. The respective values 21 days after administration were 22% and 9.5%. The main residue in muscle and fat tissues was the parent dicyclanil; CGA297107 contributed only nominally (less than 5%) to the total extractable radioactive residues.

Eight studies were performed in sheep with unlabelled dicyclanil. Three did not comply with GLP, but the five other studies were done according to present GLP standards. These studies were described in detail in the previous evaluation. All the studies were reviewed. The two most recent ones were considered to represent adequately all the relevant aspects. Depletion of residues from various tissues was analysed statistically, on the basis of data obtained up to 21 days after administration, the last time for which complete data were available and intake could be calculated. The data were pooled when possible. Linear interpolation was used to estimate the ratio of marker to total residue for measurement times between 7 and 21 days.

The statistical analysis addressed the predictability of total residues when dicyclanil was used alone or with CGA297107 as the marker residue. When the combination was used, the data were slightly less variable, but the results with the two approaches were essentially similar. The Committee considered that use of dicyclanil was preferable, for several reasons. The most important was that dicyclanil and CGA297107 must be analysed in two separate high-performance liquid chromatography (HPLC) runs, and this would place an unnecessary burden on a residue control programme.

The highest residue concentrations in muscle, liver and kidney were recorded in Merino sheep, and these were therefore considered representative of the depletion pattern. Data on depletion from fat tissue of white Alp sheep were used for a similar reason. Differences in the ratio of dicyclanil to its metabolite CGA297107 were seen in muscle tissues collected from various parts of the animals. The statistical analysis was performed with the logarithms of the concentrations and linear regression. Thereafter, statistical tolerance limits were calculated as the one-sided upper 95% confidence limit for the 95th percentile of the population. The only study that could be used to determine the ratio of total to marker residues, which is essential for determining factors to allow correction for bound residues in the various tissues, was done with Dorset sheep. As this study was limited to two measurement times, linear interpolation of the data was
used to reflect the proportions of the residues at other times. The MRLs recommended by the Committee at its fifty-fourth meeting were not consistent with this approach, and consequently different MRLs were recommended.

The methods of analysis were described in the previous evaluation. An assessment \((II, I_2)\) incorporates important information on the analytical method. The submitted data included chromatograms obtained by use of the method. The method was validated for all sheep tissues according to all the requirements of Volume VI of the rules governing medicinal products in the European Union, for both dicyclanil and its metabolite CGA297107. The Committee noted, however, that in the most recent residue depletion study significant modifications were made to the validated method, including extraction, elution and mobile phase composition, in order to improve its performance characteristics. This information was also considered in preferring the use of a single marker residue.

It should be recalled that dicyclanil and its metabolite CGA297107 are analysed by two partially separate methods. The extraction procedure is identical (for the same sample) until the final elution. Dicyclanil is then eluted with 1% isopropyl alcohol in dichloromethane, while CGA297107 is eluted with 25% isopropyl alcohol in dichloromethane. These compounds, although cations, are eluted from a strong anion exchange solid-phase extraction cartridge with organic solvents. The two fractions are injected separately into two HPLC systems. The description of the method includes a warning that the two fractions contain compounds that may interfere with the signal of dicyclanil or vice versa. The limit of quantification of the method for dicyclanil was 10 \(\mu\)g/kg and that for CGA297107 was 100 \(\mu\)g/kg for all tissues.

The additional method for determination, which was suggested as a confirmatory method in the documentation, includes an extraction procedure similar to that of the other method, which involves a C18 column for dicyclanil and an NH\(_2\) column (with a CN guard column) for analysis of CGA297107. There was good agreement between the determinative and the suggested confirmatory methods for dicyclanil, while the agreement was poor for CGA297107 in muscle and fat samples. The limit of quantification for CGA297107 was accordingly set at 100 \(\mu\)g/ml because of the presence of a large interfering peak. The suggested confirmatory method had the best chromatographic performance in terms of baseline noise and separation and, therefore, appeared best suited for routine analysis of dicyclanil.
The Committee also attempted to recalculate the TMDI resulting from the MRLs it had recommended at its fifty-fourth meeting, which were 400 μg/kg for liver and kidney, 200 μg/kg for muscle and 150 μg/kg for fat. When these MRLs were used to calculate the TMDI on the basis of selection of dicyclanil as the marker residue, the TMDI was > 680 μg per person per day (> 160% of the ADI). A precise calculation could not be made, as this would require extrapolation of the data on the ratio of marker to total residue in muscle and fat far beyond the existing database. For the same reason, the TMDI could be estimated only roughly when the sum of dicyclanil and its metabolite CGA297107 was used as the marker residue, with the same numerical values for the MRLs. The only estimate that could be made was a TMDI of > 540 μg per person per day or > 130% of the ADI.

As the Committee has attempted to propose MRLs that limit the exposure of consumers to residues, MRLs are reduced to an amount that is consistent with good practice in the use of veterinary drugs and to the extent that practical analytical methods are available. The Committee therefore based its recommendations for MRLs on residue concentrations estimated 28 days after treatment. The recommended MRLs, based on concentrations of dicyclanil, are as follows: liver and kidney, 125 μg/kg; muscle, 150 μg/kg; and fat, 200 μg/kg.

It was not possible to estimate a TMDI on the basis of the ratio of marker to total residue, since these MRLs would be reached 28–32 days after withdrawal of the dose. While sufficient data were available for these times to estimate the concentrations of parent drug and the metabolite with reasonable statistical certainty, the ratio of marker to total residue is unknown. Nevertheless, it can be assumed that, such a long time after treatment, these two compounds are the only residue of concern. Therefore, TMDIs can be estimated on the basis of the sum of dicyclanil and CGA297107 (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Food commodity</th>
<th>MRL (μg/kg)</th>
<th>Dicyclanil + CGA297107 (μg/kg)</th>
<th>Consumption (g per person per day)</th>
<th>Intake (μg per person per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>125</td>
<td>340</td>
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<tr>
<td>Sum</td>
<td></td>
<td></td>
<td></td>
<td>130</td>
</tr>
</tbody>
</table>
On the basis of consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat, using dicyclanil as the marker residue and estimating dietary intake of the sum of dicyclanil and the metabolite, the Committee estimated a TMDI of dicyclanil residues from veterinary use of 0.13 mg (31% of the ADI). A more realistic estimate of intake based on long-term intake of residues at concentrations corresponding to the geometric mean of the sum of the two compounds would give 31 μg/day or about 7% of the ADI. Use of food consumption figures in the GEMS/Food diets and the concentrations corresponding to the MRLs would result in estimates of intake of 0.18–3.6 μg per person per day or 0.04–0.85% of the ADI.

The following points were considered in setting the MRLs:

— An ADI of 0–7 μg/kg bw, based on a toxicological end-point, was recommended, which would result in a maximum daily intake of 420 μg/kg for a 60-kg person.
— The marker residue is the parent dicyclanil.
— Dicyclanil residues can be detected by HPLC at a limit of quantification of 11 μg/kg.

The Committee did not consider use of dicyclanil in lactating sheep.

3.3.3 Trichlorfon

Trichlorfon (metrifonate) was evaluated by the Committee at its fifty-fourth meeting (Annex 1, reference /4/), when it established an ADI of 0–20 μg/kg bw on the basis of a NOEL of 0.2 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in humans treated orally and applying a safety factor of 10.

Toxicological data

A re-evaluation of the ADI for trichlorfon was requested on the basis of the availability of new data that were not reviewed by the previous Committee. The Committee at its present meeting considered the results of additional studies on the pharmacokinetics of trichlorfon and on genotoxicity, reproductive toxicity, developmental toxicity, toxicity to mammalian germ cells and studies in humans.

The pharmacokinetics of trichlorfon was studied in 24 volunteers with renal disease who had various levels of impairment of renal clearance, in 34 healthy volunteers who also received magnesium- and aluminium hydroxide-containing antacids or H₂ receptor antagonists (cimetidine or ranitidine) and before and after a meal in 26 healthy volunteers. Trichlorfon
was administered as a single oral dose of 50 mg to the volunteers with renal disease, as three oral doses of 50 mg given 1 week apart to the volunteers also receiving antacids and as a single oral dose of 50 or 80 mg to the volunteers before or after a meal. The pharmacokinetics of trichlorfon was not significantly altered in any of these studies. The reported elimination half-life was approximately 2 h, similar to the value found by the Committee at its fifty-fourth meeting. In all studies, trichlorfon caused significant reductions in plasma cholinesterase activity, while erythrocyte cholinesterase activity was relatively unaffected. The authors concluded that multiple doses were required to attain significant inhibition of erythrocyte cholinesterase activity and a steady state of therapeutically relevant inhibition. The data available to the Committee were insufficient to determine whether significant species differences exist with regard to the pharmacokinetics of trichlorfon. The Committee noted that higher NOELs were observed in studies in which trichlorfon was administered in feed rather than by direct oral administration in tablets or by gavage. Therefore, differences in pharmacokinetics may result from differences in the bioavailability of the dosage form administered.

Trichlorfon has been tested in a large number of studies for genotoxicity covering a wide range of end-points, with considerable variation in the results for most end-points. Both positive and negative results were obtained in tests for bacterial mutations and for gene mutation in mammalian cells in vitro, but the results of studies of effects on chromosomes in mammalian cells in vitro (chromosomal aberrations or sister chromatid exchanges) were uniformly positive. Mostly negative results were found in assays in mammals in vivo assessed by the Committee at its fifty-fourth meeting, comprising tests for somatic cell mutations in bone marrow (sister chromatid exchange, single study, negative result), micronucleus formation (negative results in five of six studies) and chromosomal aberrations (negative results in three of five studies). Mostly negative results were also found in assays for germ cell mutagenicity in vivo evaluated by the Committee at its fifty-fourth meeting, comprising dominant lethal mutations (negative results in six of nine studies) and chromosomal aberrations in spermatogonia or spermatocytes (negative results in three of four studies). The Committee at its present meeting received further data on mutagenicity, comprising positive results in studies of sister chromatid exchange in vivo but not in vitro. Studies discussed later in this report showed that trichlorfon was a germ cell aneugen in laboratory animals in vivo. There was also limited evidence from observations in poisoned humans that trichlorfon caused aneuploidy and chromosome damage in lymphocytes. A study involving pregnant women suggested that exposure to uncertain concentrations of residues of trichlorfon in fish may have caused trisomy 21 (Down
syndrome) in their offspring as a result of germ cell aneugenicity. The Committee at its fifty-fourth meeting noted that bioassays for carcino-
genicity in rats and mice gave negative results and identified a NOEL for developmental toxicity. The Committee at its present meeting concluded that the weight of the evidence from the assays for mutagenicity in vivo indicated that trichlorfon residues in animal-derived foods would not present a carcinogenic hazard to consumers.

In a two-generation study of reproductive toxicity, trichlorfon was administered in the diet to groups of rats at concentrations providing a dose equivalent to 0, 7.5, 25 or 88 mg/kg bw per day. The parameters evaluated in adults and pups included clinical end-points, body weight, food consumption and gross and histological appearance. The body weights of F0 males and females at the highest dose were significantly decreased during the pre-mating phase, although feed consumption appeared to be unaffected by treatment. The body weights of F1 dams were decreased during gestation and lactation. The feed consumption of pups in the first and second generation at the highest dose was decreased during the lactation phase. The lactation index (live pups per litter on lactation day 21/live pups per litter on lactation day 4, after culling × 100) was significantly decreased in the first generation at the highest dose. In the second generation, significant decreases in birth index (pups born per litter/ implantation sites per litter) and mean litter size were observed at the lowest and highest doses, but there was no dose–response relationship. The body weight of pups at the highest dose was decreased in both generations on day 21. No abnormalities were observed at gross and histological examination of adults and pups of either generation. At termination, significant decreases in plasma and brain cholinesterase activity were reported in F0 females at all doses. In addition, erythrocyte cholinesterase activity was significantly decreased in females and males at the two higher doses. Brain cholinesterase activity was significantly decreased in F0 males at the highest dose. At termination of the F1 generation, plasma cholinesterase activity was significantly decreased in adult males and females at the two higher doses. Erythrocyte cholinesterase activity was significantly decreased in females at all doses and in males at the highest dose. Neonatal erythrocyte cholinesterase activity was significantly decreased in F1 males at the two higher doses on day 4 of lactation. On day 21 of lactation, significant decreases in brain and plasma cholinesterase activity were observed in male and female pups at the highest dose. In the F2 generation, significant decreases in plasma cholinesterase activity were observed in male and female pups, and significant decreases were found in brain cholinesterase activity in females at the highest dose and in males at the two higher doses on day 21 of lactation. A NOEL could not be
identified in this study, as significantly decreased plasma and brain cholinesterase activities were seen at term in adult F₀ females at the lowest dose and significantly decreased erythrocyte and brain cholinesterase activities in F₁ adult females at the lowest dose. The Committee noted that the LOEL in this study (7.5 mg/kg bw per day) was lower than the NOEL of 30 mg/kg bw per day identified in a three-generation study of reproductive toxicity in rats by the Committee at its fifty-fourth meeting. It also noted, however, that cholinesterase inhibition was not evaluated in that study. Had that been done, it is reasonable to assume that the NOEL for that study would have been lower than 30 mg/kg bw per day. This assumption is supported by the NOEL of 5 mg/kg bw per day for inhibition of erythrocyte cholinesterase activity identified by the Committee at its fifty-fourth meeting in a 16-week study of toxicity in rats treated orally. On the basis of these considerations and the fact that cholinesterase inhibition was the most sensitive effect in offspring in the present study, with an NOEL of 7.5 kg/kg bw per day, the Committee concluded that the reproductive toxicity of trichlorfon in rats had been adequately assessed.

In a study of developmental toxicity in guinea-pigs, designed to evaluate brain hypoplasia in offspring exposed in utero, trichlorfon was administered either by stomach tube or by subcutaneous injection. Oral doses ranging from 25 to 200 mg/kg bw per day were administered to groups of rats on days 40–44 of gestation in various regimens. Clinical signs of toxicity typical for this substance, including hypersalivation and hindlimb weakness, were observed in dams given trichlorfon at 100, 150 or 200 mg/kg bw. The NOEL for brain hypoplasia in offspring was 50 mg/kg bw.

In a study of the cytogenetic and developmental effects of trichlorfon on pre-implantation, mid-gestation and near-term mouse embryos and fetuses in vivo, groups of mice were given an intraperitoneal injection of 100 or 200 mg/kg bw 6 h after presumed conception. Developmental outcomes and micronucleus formation during the pre-implantation phase were assessed on day 3 of gestation, and developmental and aneugenic outcomes during the mid-gestation period were assessed on day 9 of gestation. On day 17 of gestation, the embryos were removed, sexed, weighed and examined for external malformations. No clinical signs of toxicity were observed in the treated mice. The mean cell number of embryos in both treated groups was significantly lower than that in the control group, and the mean number of micronuclei was significantly increased in both treated groups compared with controls. The number of live embryos per dam was significantly lower among those given 100 mg/kg bw than in controls. The mean number of somites in the trichlorfon-treated groups was significantly lower than in controls. Significantly more fetuses with abnormal numbers
of chromosomes were found to be associated with trichlorfon treatment. The incidence of external malformations and the body weights of male and female fetuses in the trichlorfon-treated groups were comparable to those of controls. This study provides evidence that exposure to trichlorfon around the time of fertilization could result in induction of micronuclei, aneuploidy and developmental retardation in embryos from pre-implantation to mid-gestation. Embryos with micronuclei or aneuploidy may no longer show abnormalities at term. The Committee noted that these effects resulted from intraperitoneal injection of doses 5000 to 10 000 times greater than the current ADI established for orally administered trichlorfon by the Committee at its fifty-fourth meeting.

The effects of trichlorfon on fertilization, spindle morphology and chromosomal segregation were studied in mouse oocytes exposed in vitro to a concentration of 50 µg/ml. Aberrant spindles were observed in maturing oocytes after 8 h of exposure, and the chromosomes appeared to be incapable of proper alignment at the equator. After 16 h, most of the treated oocytes had highly aberrant spindles, and the chromosomes were frequently unaligned and located at different distances from the poles. Such effects on spindles and chromosome alignment could have aneugenic effects on fertilized eggs and embryos. The Committee noted that effects on spindles leading to aneuploidy have thresholds. The Committee also noted that, assuming 100% bioavailability, systemic exposure to trichlorfon resulting from consumption of the entire ADI would be orders of magnitude lower than the dose used in this study. Exposure to trichlorfon at the ADI would therefore pose a negligible risk to human oocytes.

Aneuploidy induction was studied in sperm cells collected from groups of male mice 22 days after a single intraperitoneal injection of trichlorfon at a dose of 200, 300 or 400 mg/kg bw. A significant, dose-related increase in the percentage of sperm cells with an extra chromosome was observed at all doses. On the basis of this experiment, the Committee concluded that trichlorfon is a male mouse germ-cell aneugen in vivo. The Committee noted that these effects resulted from intraperitoneal injection of doses several orders of magnitude higher than the current ADI for trichlorfon established by the Committee at its fifty-fourth meeting.

The maximal effects of trichlorfon on soluble neuropathy target esterase activity and regional distribution of neuropathy target esterase and acetylcholinesterase were studied in brain and spinal cord of hens given trichlorfon at a single intravenous injection of 200 mg/kg bw. Peak inhibition of neuropathy target esterase activity occurred 6 h after dosing and ranged from 15 to 44%. No signs of delayed neuropathy were found during the 28-day observation period in four treated hens. Having reviewed several
contemporary studies, the Committee at its fifty-fourth meeting also concluded that trichlorfon did not cause delayed neuropathy in hens.

A cluster of congenital anomalies was identified in a Hungarian village between 1989 and 1990. Of 15 live births, 11 (73%) were affected by abnormalities, and six were twin births. Four of the 11 affected infants had trisomy 21 (Down syndrome). Nine different physical abnormalities were observed. The mothers of all the affected infants reported having eaten fish during pregnancy. Several ponds around the village used for fish farming had been treated with a trichlorfon formulation at a level of 500 mg/l. The average concentrations of trichlorfon measured or estimated in the types of fish consumed ranged from 0.15 to 100 mg/kg. The exposure of fathers to trichlorfon was not evaluated in this study. This is the only known report of reproductive effects in humans possibly associated with oral exposure to trichlorfon, despite its widespread use as an anthelmintic.

The Committee acknowledged that the results of this study were not conclusive and provided limited evidence of a possible association between birth defects in humans and oral intake of trichlorfon in food. In addition, the published report did not include confirmation of the magnitude or frequency of intake or even whether intake had occurred. The Committee at its fifty-fourth meeting evaluated studies of developmental toxicity with trichlorfon conducted in four animal species. In these studies, teratogenic effects were seen only at very high, maternally toxic doses. In addition, as multigeneration studies of reproductive toxicity did not provide evidence of paternally transmitted teratogenicity, the Committee considered that the effect on exposed males had been assessed. The Committee at its present meeting reviewed the assessment by the Joint Meeting on Pesticide Residues in 1993 (JMPR) of dichlorvos, the major metabolite of trichlorfon. That Meeting concluded that dichlorvos was not teratogenic in mice, rats or rabbits, even at doses that were toxic to maternal animals. In addition, at 12 mg/kg bw per day, dichlorvos had no reproductive effects in rats in a three-generation study. On the basis of these considerations, the Committee concluded that the information from the study in humans would not significantly affect its risk assessment of trichlorfon.

Chromosomal effects were studied in the lymphocytes of 31 people who had attempted suicide by taking unknown doses of trichlorfon. There appeared to be an increase in per cent aneuploidy in blood samples collected 3–6, 30 and 180 days after the incidents. An increase was also found in the rate of chromatid and chromosome-type aberrations. The Committee concluded that the intake that had resulted in these effects far exceeded the ADI established for trichlorfon by the Committee at its fifty-fourth meeting.
The additional information reviewed by the Committee for this re-evaluation of trichlorfon included further information on its pharmaco-kinetics and genotoxic, reproductive and developmental toxicity. In addition, a NOEL for teratogenicity in guinea-pigs was established. The information did not provide evidence that any of these effects was more sensitive than inhibition of acetylcholinesterase activity, and the Committee concluded that inhibition of acetylcholinesterase activity is the most relevant end-point for establishing an ADI for trichlorfon.

The Committee at its fifty-fourth meeting established the ADI for trichlorfon on the basis of a NOEL of 0.2 mg/kg bw per day in a study of human volunteers with Alzheimer disease treated with trichlorfon. In this study, volunteers were given a loading dose of 0.5 mg/kg trichlorfon daily for 2 weeks, followed by a maintenance dose of 0.2 mg/kg per day for 8 weeks. The Committee at its fifty-fourth meeting concluded that the maintenance dose had not significantly enhanced the inhibition of erythrocyte cholinesterase activity established in patients by the loading dose. Therefore, it concluded that the maintenance dose was the NOEL in this study. Because the NOEL was derived from a study in humans, a safety factor of 10 was applied to the NOEL to derive the ADI.

The present Committee re-examined the basis on which it had established the ADI for trichlorfon at its fifty-fourth meeting and concluded that the dose it had identified then as the NOEL was nevertheless effective in maintaining the steady-state level of inhibition of erythrocyte cholinesterase activity and was therefore more appropriately considered a LOEL. The Committee concluded, however, that an ADI could be derived from this study by applying an additional factor of 10 to this LOEL. This conclusion is supported by supplemental information that included a linear dose extrapolation of data from a study of 27 volunteers with Alzheimer disease, who were treated with loading doses of 1.5–4 mg/kg bw per day for 6 days, followed by maintenance doses of 0.25–1 mg/kg bw per day for 15 days. The linear extrapolation resulted in an estimated NOEL for inhibition of cholinesterase activity in the range 0.1–0.2 mg/kg per day. This provides further support that the NOEL for inhibition of erythrocyte cholinesterase activity in humans is very close to 0.2 mg/kg bw per day. Furthermore, the Committee recalled that a clear NOEL of 0.2 mg/kg per day for inhibition of erythrocyte cholinesterase activity was established in a 10-year study of toxicity in monkeys treated orally, which it had evaluated at its fifty-fourth meeting. If that study had been selected as the basis for setting the ADI, a safety factor of 100 would have been applied, resulting in an ADI of 0–2 μg/kg bw. The present Committee thus amended the ADI for trichlorfon
from 0–20 μg/kg bw to 0–2 μg/kg bw on the basis of the LOEL of 0.2 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in humans treated orally and a 100-fold safety factor.

Residue data

The ADI of 0–2 μg/kg bw established by the Committee at its present meeting for trichlorfon provides for a maximum intake of 120 μg of residues of trichlorfon for a 60-kg person. At its fifty-fourth meeting, the Committee recommended an MRL of 50 μg/kg for milk, equivalent to 75 μg for consumption of 1.5 kg of milk. The values for muscle, liver, kidney and fat of 50 μg/kg should serve as guidelines for monitoring residues by national authorities, as no residues of trichlorfon were detected in the residue depletion studies reviewed by the Committee. Further, the Committee at its fifty-fourth meeting considered that no residues would be found in muscle, liver, kidney or fat at the limit of quantification of the available analytical methods. The Committee affirmed those considerations.

In estimating theoretical maximum daily intakes (TMDIs) of trichlorfon, only the MRL for milk should be considered. On this basis, the TMDI is 75 μg, equivalent to about 62% of the ADI.

3.4 Production aid

3.4.1 Carbadox

Carbadox is used to promote the growth of pigs and as an antibacterial drug for prevention of dysentery in pigs. Carbadox was evaluated by the Committee at its thirty-sixth meeting (Annex 1, reference 92), but an ADI could not be established because of evidence for the genotoxicity and carcinogenicity of carbadox and its metabolites desoxy carbadox and hydrazine. The Committee at its thirty-sixth meeting noted that the residues of carcinogenic concern were not detectable when the concentrations of the non-carcinogenic residue quinoxaline-2-carboxylic acid (QCA) were at or below 0.03 mg/kg in pig liver and 0.005 mg/kg in pig muscle. Therefore, the Committee recommended MRLs of 0.03 mg/kg in liver and 0.005 mg/kg in muscle of pigs measured as QCA.

Toxicological data

The Committee considered data on the toxicology and residues of carbadox and its metabolites in porcine tissues that had been generated since the previous evaluation of this substance.
In a study of developmental toxicity, carbadox was administered orally to pregnant rats at a dose of 0, 10, 25, 50 or 100 mg/kg bw per day on days 8–15 of gestation. None of the treated dams died. Maternal body-weight gain was significantly decreased in a dose-related manner at all doses; weight gain recovered after cessation of treatment, except in animals at the highest dose. The animals were killed on day 21 of gestation, and their fetuses were removed surgically. Carbadox was embryotoxic and fetotoxic, as indicated by a dose-related reduction in fetal body weight, which was statistically significant at doses of 25 mg/kg bw per day and higher. The number of live fetuses and the resorption rate in dams at doses of 50 mg/kg bw per day or less were not different from those of the control group. Embryolethality occurred at the highest dose, the percentage of late resorptions being 82% and the number of live pups being reduced by more than 80% when compared with controls. At the highest dose, carbadox induced external, skeletal and internal malformations at rates of 47%, 45% and 28%, respectively. The abnormalities recorded were short tail, kinky tail, brachygnathia, ectrodactyly, club foot, generalized oedema, fused vertebrae and hydrocephaly. Carbadox was considered to be embryotoxic and fetotoxic as a consequence of its strong maternal toxicity and to be teratogenic in rats. The NOEL was 10 mg/kg bw per day for embryotoxicity and 50 mg/kg bw per day for teratogenicity. An NOEL for maternal toxicity could not be identified. The Committee noted that the NOELs for developmental toxicity in this study were well above the NOEL of 2.5 mg/kg bw per day found in the studies of reproductive toxicity in rats previously evaluated by the Committee.

No new experimental data on the metabolism of carbadox or on the genotoxicity or carcinogenicity of carbadox and its metabolites were provided. The Committee was aware of an evaluation based on linear extrapolation to estimate a “virtually safe dose” for carbadox and its metabolites of carcinogenic concern. This estimate was made on the basis of the incidence of hepatic tumours in rats in long-term studies of carcinogenicity previously evaluated by the Committee. The evaluation resulted in codification of tolerance for residues of carbadox and the metabolites of carcinogenic concern in edible tissues and their risks to the consumer. The Committee did not consider this approach appropriate owing to the substantial inherent uncertainties involved.

The results of a recent study of residues of carbadox in pigs indicated longer persistence of substantial amounts of the carcinogenic metabolite desoxycarbadox in liver at the time when the concentration of the marker residue, QCA, fell below the MRL of 0.03 mg/kg and to the end of the experiment 15 days after withdrawal.
Carbadox was reviewed by the present Committee primarily on the basis of new information on residue levels, which indicated that the metabolite desoxycarbadox was present in edible tissues even at the end of the 15-day experimental withdrawal period. The Committee confirmed that the information previously submitted indicated that both carbadox and desoxycarbadox should be regarded as carcinogens that act by a genotoxic mechanism. Although the Committee was aware that linear extrapolation has been used to estimate a “virtually safe dose” for carbadox, the Committee concluded that it was not possible to identify a dose of carbadox that poses an acceptable risk to consumers. The Committee therefore did not establish an ADI for carbadox.

**Residue data**

At the Thirteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (5), Canada requested that carbadox be given priority for review at the next meeting of the Joint Expert Committee on Food Additives, as it could be predicted from data on residues that carbadox and desoxycarbadox might be present in tissues from pigs that had not been withdrawn from treatment before slaughter. The Codex Committee accepted the request, and carbadox was placed on the agenda of the Expert Committee at its sixtieth meeting. The Joint FAO/WHO Secretariat requested submission of all relevant toxicological and residue data that had been generated since the Committee’s evaluation at its thirty-sixth meeting, including analytical methods for detecting the parent drug and metabolites in porcine tissues.

As noted above, no new data on the genotoxicity or carcinogenicity of carbadox and its metabolites had been generated since the previous evaluation, and the Committee was again unable to establish an ADI. New studies were, however, provided on depletion of residues of carbadox in pig liver, muscle, fat and skin. One of the studies, which is ongoing and subject to further evaluation, covers the first 15 days after withdrawal of medicated feed and provides detailed information on depletion of the carcinogenic residues. The results significantly change the information base from that available at the time of the first evaluation.

In reaching its decision on MRLs for carbadox, the Committee at its thirty-sixth meeting took various factors into consideration, including the following, which are now fully or partially invalid:

- The Committee concluded that carbadox and desoxycarbadox could be detected in tissues only for the first 72 h after treatment, and their concentrations 28 days after withdrawal were negligible. The new factor
is the availability of a new HPLC–MS/MS method with limits of quantification of 50 and 30 ng/kg for carbadox and desoxycarbadox, respectively. With the improved performance of the method, carbadox could be measured quantitatively in liver only up to 48 h, but desoxy-carbadox was present in quantifiable concentrations until the end of the study, 15 days after the last administration of medicated feed.

— The Committee at its thirty-sixth meeting concluded that more than 90% of the total residues in tissues were bound and could not be extracted 28 days after withdrawal. It concluded that the bound residues in pig liver 28 days after treatment would not represent a risk for consumers. With the analytical procedures available at that time, QCA was the only carbadox metabolite that could be identified in liver from pigs treated according to good practice in the use of veterinary drugs. The methods described in the new study included treatment of samples with digestive enzymes (United States Pharmacopeia [USP] systems that mimic gastric and intestinal fluids). With these techniques, the amount of desoxy carbadox that could be released from liver tissues was increased by two- to fourfold, and the possibility cannot be excluded that desoxy carbadox could be released at times beyond the 15 days of the duration of the new study.

— The Committee at its thirty-sixth meeting also concluded that the amount of QCA extracted by alkaline hydrolysis was less than 30 µg/kg 28 days after withdrawal. Practical analytical methods were available for measuring QCA at concentrations down to 30 µg/kg in liver and 5 µg/kg in muscle. On the basis of studies on the toxicity of QCA and on the metabolism and depletion of carbadox and the nature of the compounds released from bound residues, the Committee concluded that concentrations of residues resulting from use of carbadox in pigs were acceptable, provided those of QCA were below 30 µg/kg in liver and below 5 µg/kg in muscle. The Committee recommended MRLs of 30 µg/kg in liver and 5 µg/kg in muscle of pigs, based on the concentrations of, and expressed as, QCA. While the new studies confirmed the good correlation between the concentrations of QCA and desoxy carbadox in liver and also confirmed the time required to deplete QCA to less than 30 µg/kg, they also showed that desoxy carbadox is still present in liver when the concentrations of QCA have reached the MRL. Calculation of the relationship between the concentrations of the two metabolites by the Committee by linear regression of the logarithms of the concentrations showed that 30 µg/kg of QCA in liver corresponded to approximately 4 µg/kg of desoxy carbadox. The tolerance limits for the concentration of desoxy carbadox were several times higher owing to the wide variation of the data. Therefore, QCA
is not a suitable marker for monitoring carcinogenic metabolites of carbadox in liver to comply with the MRL recommended by the Committee at its thirty-sixth meeting, and QCA does not ensure the absence of carcinogenic residues. QCA is also not a suitable marker for ensuring the absence of carcinogenic residues in muscle.

Two new studies were provided, supplying information on depletion of residues of carbadox in pig liver and muscle. In one, QCA residues were measured in the liver of pigs fed medicated feed containing carbadox in combination with oxytetracycline, after a withdrawal period of up to 42 days. In the other study, QCA, carbadox and desoxycarbadox residues were measured during the first 15 days after administration of medicated feed containing 55 mg/kg; this study provided detailed information on depletion of the carcinogenic residues.

The study to determine depletion of QCA residue in pig liver after administration of carbadox and oxytetracycline involved 35 pigs fed a diet containing carbadox at 28 mg/kg (25 g/l) for 28 days and in combination with oxytetracycline at 880 mg/kg for an additional 14 days. Five animals were killed at each of seven times, and their livers were analysed for QCA by gas chromatography with electron capture detection (GC–EC) (limit of quantification [LOQ], 5 μg/kg). The mean QCA concentrations were 130 μg/kg at 0 days, 41 μg/kg at 7 days, 29 μg/kg at 14 days, 7 μg/kg at 21 days, 3 μg/kg at 28 days, 2 μg/kg (one value) at 35 days and 2 μg/kg at 42 days.

In the short-term study to investigate depletion of residues during the first 15 days after withdrawal of the drug, 34 pigs were fed a diet containing the maximum approved concentration of 50 g/l of feed (55 mg/kg) for 14 days. Three animals were killed 3, 6, 9, 12 and 24 h and 2, 4, 7, 10 and 15 days after withdrawal, and the concentrations of carbadox, desoxycarbadox and QCA were determined either directly in tissues, in whole tissue samples after incubation with USP simulated gastric fluid (pepsin) and USP simulated intestinal fluid (pancreatin) or in the supernatant of samples after treatment with simulated digestive fluids. QCA was determined by GC-ECD. Residues of carbadox and desoxycarbadox were determined quantitatively by liquid chromatography with tandem mass spectroscopy (LC-MS/MS) after extraction with acetonitrile. After enzymic treatment of the samples, residues of carbadox, desoxycarbadox and QCA were determined by LC-MS/MS after extraction with ethyl acetate. The reported LOQs were 50 ng/kg for carbadox, 30 ng/kg for desoxycarbadox and 15 μg/kg for QCA.
In this study, QCA was the main metabolite in liver, followed by desoxycarbadox. Carbadox in liver depleted from 420 ng/kg at 0 h to 50 ng/kg by 2 days after withdrawal, desoxycarbadox depleted from 10 500 ± 2200 ng/kg at 0 h to 140 ± 56 ng/kg 15 days after withdrawal, and QCA depleted from 190 000 ± 69 000 ng/kg at 0 h to 18 000 ± 2000 ng/kg 15 days after withdrawal (standard deviations are included to indicate the wide variation of the results).

Pretreatment of the samples with digestive fluids increased the amounts of carcinogenic residues found in all tissues. In liver, the concentration of carbadox increased to 620 ± 160 ng/kg at the time of withdrawal but was not quantifiable 10 days later. The concentration of desoxycarbadox increased by more than fourfold when the samples were treated with intestinal fluid, and large quantities were present 15 days after withdrawal (35 000 ± 4400 ng/kg at 0 h, 3000 ± 2400 ng/kg 15 days after withdrawal).

QCA was detected in muscle tissue only in two samples taken 0 and 3 h after withdrawal. The concentrations of carbadox and desoxycarbadox decreased steeply in all muscle samples during the first few hours after withdrawal of medicated feed, and carbadox was not detectable 12 h after withdrawal. The only residue that was quantifiable up to the end of the study (15 days) was desoxycarbadox, which was found in very small quantities (74 ng/kg in tissue, 43 ng/kg after intestinal fluid treatment). QCA was not detected in skin or fat. Carbadox was not detectable in skin tissue 10 days after withdrawal, and desoxycarbadox was the only residue quantifiable 15 days after withdrawal, occurring in small quantities (< 100 ng/kg). Neither carbadox nor desoxycarbadox was quantifiable 7 days after withdrawal. One sample of kidney from an animal in which the values in liver were the highest at each time was analysed 0, 6, 12, 24, 48 and 96 h after withdrawal. QCA was not detected 24 h after withdrawal, and no carbadox was found 48 h after withdrawal. Desoxycarbadox depleted quickly, from 22 000 ng/kg at 0 h to 420 ng/kg 96 h after withdrawal.

In a study on carbadox residues, 34 cross-bred pigs (17 gilts and 17 barrows) were given feed containing carbadox at 55 mg/kg for 28 days. Muscle and liver were collected from each animal for determination of QCA residues by the regulatory GC–EC method (LOQ, 5 µg/kg). The concentration of residue in muscle was below the LOQ. Those of QCA were 52 µg/kg 14 days after withdrawal, 29 µg/kg at 21 days, 18 µg/kg at 28 days, 11 µg/kg at 35 days, 11 µg/kg at 42 days and 11 µg/kg at 49 days. The concentration of QCA was less than 30 µg/kg 28 days after withdrawal.
Analytical methods

Two methods of determination were used in the three new residue studies considered by the Committee. The recognized regulatory method, based on GC–ECD and accepted by the Committee at its thirty-sixth meeting, was used in two studies to determine residues resulting from treatment of pigs at the dose rate stipulated on the label and at one-half that rate. This method involves alkaline hydrolysis digestion to release bound residues and conversion of any carbadox parent compound or related metabolites present in the tissue to the marker residue, QCA. The method was used for liver in the one-half dose study and for liver and muscle in the study at the recommended dose rate, with a reported LOQ of 0.005 mg/kg. This analytical method has been used routinely in many regulatory laboratories for over a decade.

A new method based on liquid chromatography–atmospheric pressure chemical ionization with tandem mass spectrometry (LC–APCI–MS/MS) was developed for use in a recent study to measure the concentrations of carbadox, desoxycarbadox and QCA. The method had better specificity than the regulatory GC–ECD method. Quantification is based on measurement of a product ion separated in the second stage of MS/MS after fragmentation of a precursor ion formed from the parent molecule in the first stage of MS/MS. A linear response was found across the analytical range, with reported LOQs of 50 ng/kg for carbadox, 30 ng/kg for desoxycarbadox and 15 µg/kg for QCA. The reported LOQs may be conservative, as they were the lowest concentrations at which the method was tested to meet the requirements of precision and recovery, but they do not necessarily represent the lowest possible concentrations that might be found experimentally. The method was tested on liver, kidney, muscle, skin and fat, with acceptable recoveries from all tissues except muscle at or above the concentrations noted above. The recovery from muscle was in the range of 400% at concentrations < 1000 ng/kg, suggesting that the external calibration used was unsuitable due to matrix effects. Enhancement due to the matrix may occur in analytical methods for traces based on mass spectral detection. The method offers excellent capability for the determination of trace concentrations of carbadox and desoxycarbadox. It supplements the GC–ECD regulatory method for determination of total residues of toxicological concern and allows determination of the contribution of parent carbadox and desoxycarbadox to the residues measured as QCA. Additional product ions are available, which, while not used for quantification, may provide additional information for confirmation. Data to support recognition of the method as a confirmatory method were not provided for evaluation.
The LC–MS/MS method was also developed for analysis of supernatants derived from digestion of tissues containing carbadox residues with two enzyme systems considered to be representative of gastrointestinal processes. After a 4-h digestion with the enzymes, both carbadox and desoxycarbadox were found to be unstable when treated with pepsin, but pancreatin had little effect on either compound. Digestion with the addition of liver to the fluid also showed the instability of carbadox and desoxycarbadox with pepsin treatment and a decrease in the concentration of desoxycarbadox after pancreatin treatment. QCA recovery was not affected by either treatment.

The results of the short-term depletion study were also used to establish a relationship between the concentration of the marker metabolite QCA in the target tissue, liver, and of the carcinogenic residues carbadox and desoxycarbadox in liver and muscle, respectively. A good linear relationship was found between the logarithms of the concentrations of QCA and desoxycarbadox in liver, but no such relationship was determined for muscle tissue. For a concentration of QCA in liver of 30 µg/kg, the average concentration of the carcinogenic residue desoxycarbadox in liver was estimated by regression analysis to be about 4 µg/kg.

Conclusions

— The new data confirm that carcinogenic residues, in particular desoxycarbadox, are present in edible tissues during depletion of parent carbadox. The relatively long persistence of the residues was a new finding. The results also show that, after administration of the highest recommended dose of 55 mg/kg in feed, QCA depletes to below the MRL for liver recommended by the Committee at its thirty-sixth meeting within a short time (approximately 17 days on the basis of the upper limit of the 95% confidence interval on the 99th percentile).

— The experiments conducted with digestive enzymes showed that the true concentrations of the carcinogenic metabolites in tissues cannot yet be estimated with certainty, since an unknown portion of the releasable residue is destroyed during incubation with the enzymes. Therefore, the total residue measured in the supernatant after enzyme digestion and in the remaining tissue represents a lower estimate of the total present in the tissue. The fraction of this residue that could be considered to be bioavailable might be lower, but this value cannot be determined with reasonable certainty.

— As the Committee was unable to allocate an ADI for carbadox, there is no accepted reference point for comparison with the new data on residues. Therefore, on the basis of the new data, the MRL for QCA
recommended by the Committee at its thirty-sixth meeting was not supported for residues of carbadox of toxicological concern in liver.

— The MRL of 5 µg/kg recommended by the Committee at its thirty-sixth meeting for QCA in muscle is not supported by the new data. As desoxycarbadox was found at all times up to 15 days but QCA was found in only two samples collected 0 and 3 h after withdrawal, the relationship between the concentrations of QCA and desoxycarbadox is not known.

— After reviewing the new studies, the Committee could not determine the amounts of residues of carbadox and its metabolites in food that represented an acceptable risk to consumers. The Committee decided to withdraw the MRLs of carbadox recommended by the Committee at its thirty-sixth meeting.

4. **Recommendations**

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

2. In view of the continuing need for evaluations of veterinary drugs, meetings of the Joint FAO/WHO Expert Committee on Food Additives should be held regularly for that purpose.

**Acknowledgement**

The Committee wished to thank E. Heseltine, St Léon-sur-Vézère, France, for her assistance in the preparation of the report.

**References**


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


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


55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives).* FAO Food and Nutrition Paper, No. 17, 1980.


58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.


121. Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, No. 41/8, 1996.


Annex 2
Recommendations on compounds on the agenda

Antimicrobial agents

Neomycin

Acceptable daily intake: The ADI of 0–60 µg/kg bw (established at the forty-seventh meeting of the Committee (WHO TRS 876, 1998)) was maintained.

Residue definition: Neomycin

Recommended maximum residue limits (MRLs)*

<table>
<thead>
<tr>
<th>Species</th>
<th>Liver (µg/kg)</th>
<th>Kidney (µg/kg)</th>
<th>Milk (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>500</td>
<td>10 000</td>
<td>1500</td>
</tr>
</tbody>
</table>

* The MRLs of 500 µg/kg for cattle muscle and fat and all other MRLs recommended at the forty-seventh meeting of the Committee (WHO TRS 876, 1998) were maintained.

Flumequine

Acceptable daily intake: The ADI established at the forty-eighth meeting of the Committee (WHO TRS 879, 1998) was withdrawn.

Residues: The MRLs for cattle, pigs, sheep, chickens and trout established at previous meetings (WHO TRS 879, 1998; WHO TRS 900, 2001) were withdrawn.

Antiprotozoal agent

Imidocarb

Acceptable daily intake: 0–10 µg/kg bw (established at the fiftieth meeting of the Committee (WHO TRS 888, 1999))

Residue definition: Imidocarb free base
### Recommended maximum residue limits (MRLs)

<table>
<thead>
<tr>
<th>Species</th>
<th>Fat (µg/kg)</th>
<th>Kidney (µg/kg)</th>
<th>Liver (µg/kg)</th>
<th>Muscle (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>50</td>
<td>2000</td>
<td>1500</td>
<td>50</td>
</tr>
</tbody>
</table>

### Insecticides

**Deltamethrin**

Intake considerations: The Joint FAO/WHO Expert Meeting on Pesticide Residues performed a dietary risk assessment and estimated that the theoretical intake of deltamethrin residues from pesticide use would account for 25% of the ADI, equivalent to 150 µg (FAO Plant Production and Protection Paper No. 172, 2002). The sum of the theoretical concentrations of deltamethrin residues from use as a veterinary drug and as a pesticide use would be no more than 415 µg, equivalent to 68% of the ADI.

Residues: The Committee affirmed that the MRLs recommended at the fifty-second meeting (WHO TRS 893, 2000) were compatible with the ADI.

**Diclofenac**

Acceptable daily intake: 0–7 µg/kg bw (established at the fifty-fourth meeting of the Committee (WHO TRS 900, 2001))

Residue definition: Diclofencar

<table>
<thead>
<tr>
<th>Species</th>
<th>Muscle (µg/kg)</th>
<th>Liver (µg/kg)</th>
<th>Kidney (µg/kg)</th>
<th>Fat (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>150</td>
<td>125</td>
<td>125</td>
<td>200</td>
</tr>
</tbody>
</table>
**Trichlorfon (metrifonate)**

Acceptable daily intake: The Committee amended the ADI for trichlorfon from 0–20 μg/kg to 0–2 μg/kg bw.

Residues: The Committee confirmed the MRL for cows’ milk and the guidance levels for muscle, liver, kidney and fat of cattle recommended at the fifty-fourth meeting (WHO TRS 900, 2001).

**Production aid**

**Carbadox**

Acceptable daily intake: The Committee confirmed the opinion, expressed at its thirty-sixth meeting (WHO TRS 799 1990), that an ADI could not be established.

Residues: The Committee decided to withdraw the MRLs of carbadox recommended at the thirty-sixth meeting (WHO TRS 799 1990).
CORRIGENDA


Page 21, Figure 1:
Row 6, second box ('Do data from therapeutic use of the drug class in humans or in model systems...'), change 'No' to 'Yes' and 'Yes' to 'No'.

Page 26, 'Maximum residue limits':
line 3: Replace 'The ADI of 0–30 mg/kg bw' by 'The ADI of 0–30 µg/kg bw'.
line 13: Replace 'temporary MRL of 50 mg/kg' by 'temporary MRL of 50 µg/kg'.
line 17: Replace 'of 75 mg' to 'of 75 µg'.

Page 27, line 3:
Replace 'MRL of 200 mg/kg' by 'MRL of 200 µg/kg'.

Page 28:
'Maximum residue limits', second paragraph, line 7: Replace 'MRL of 200 mg/kg' by 'MRL of 200 µg/kg'.
'3.2.3 Lincomycin', second paragraph, line 2: Replace 'an ADI of 0–30 mg/kg bw' by 'an ADI of 0–30 µg/kg bw'.

Page 29, Table 1, second column heading:
Replace 'MRL (mg/kg)' by 'MRL (µg/kg)'.

Page 31:
'Recommended maximum residue limits'. line 3: Replace 'The ADI of 0–30 mg/kg bw' by 'The ADI of 0–30 µg/kg bw'.
Table 2, second column heading: Replace 'MRL (mg/kg)' by 'MRL (µg/kg)'.

Page 33, '3.2.5 Oxytetracycline', line 3:
Replace 'a group ADI of 0–30 mg/kg bw' by 'a group ADI of 0–30 µg/kg bw'.

Page 35, '3.2.6 Thiamphenicol':
line 3, replace 'a temporary ADI of 0–6 mg/kg' by 'a temporary ADI of 0–6 µg/kg'.
line 5, replace 'temporary MRLs of 40 mg/kg' by 'temporary MRLs of 40 µg/kg'.
line 10, replace 'an ADI of 0–5 mg/kg' by 'an ADI of 0–5 µg/kg'.
line 14, replace 'of 50 mg/kg' by 'of 50 µg/kg'.
line 15, replace '100 mg/kg in liver and 500 mg/kg in kidney, and, for fish, of 50 mg/kg' by '100 µg/kg in liver and 500 µg/kg in kidney, and, for fish, of 50 µg/kg'.
Page 45:
line 4, replace ‘which are 50 mg/kg for cattle and 20 mg/kg for sheep’ by ‘which are 50 μg/kg for cattle and 20 μg/kg for sheep’.
line 10, replace ‘400 mg/day’ by ‘400 μg/day’.

‘3.3.3 Phoxim’:
line 2, replace ‘an ADI of 0–4 mg/kg bw’ by ‘an ADI of 0–4 μg/kg bw’.
line 3, replace ‘MRLs of 20 mg/kg in muscle, liver and kidney and 400 mg/kg’ by ‘MRLs of 20 μg/kg in muscle, liver and kidney and 400 μg/kg’.

Page 47:
Second paragraph:
line 8, replace ‘35, 31 and 31 mg/kg’ by ‘35, 31 and 31 μg/kg’.
line 12, replace ‘75, 85 and 89 mg/kg’ by ‘75, 85 and 89 μg/kg’.

‘Methods of analysis’, second paragraph:
line 8, replace ‘2.0 mg/kg in liver, 3.0 mg/kg in kidney, 5.5 mg/kg in muscle’ by ‘2.0 μg/kg in liver, 3.0 μg/kg in kidney, 5.5 μg/kg in muscle’.
line 9, replace ‘3.0 mg/kg in fat. The limit of quantification was validated at 25 mg/kg’ by ‘3.0 μg/kg in fat. The limit of quantification was validated at 25 μg/kg’.
line 10, replace ‘at 200 mg/kg in fat’ by ‘at 200 μg/kg in fat’.

Page 48:
line 4, replace ‘set at 350 mg/kg’ by ‘set at 350 μg/kg’.

‘Maximum residue limits:
line 3, replace ‘The ADI of 0–4 mg/kg bw’ by ‘The ADI of 0–4 μg/kg bw’.
line 17, replace ‘of 50 mg/kg in muscle, 50 mg/kg in liver, 50 mg/kg in kidney’ by ‘of 50 μg/kg in muscle, 50 μg/kg in liver, 50 μg/kg in kidney’.
line 18, replace ‘400 mg/kg in fat, expressed as phoxim. Temporary MRLs of 50 mg/kg’ by ‘400 μg/kg in fat, expressed as phoxim. Temporary MRLs of 50 μg/kg’.
line 19, replace ‘50 mg/kg in liver, 50 mg/kg in kidney, 400 mg/kg in fat and 10 mg/kg’ by ‘50 μg/kg in liver, 50 μg/kg in kidney, 400 μg/kg in fat and 10 μg/kg’.

Page 65:
‘Insecticides, Cyhalothrin’
line 1, replace ‘Acceptable daily intake: 0–2 mg/kg bw’ to ‘Acceptable daily intake: 0–2 μg/kg bw’.
Footnote b, line 3: replace ‘limit of quantification of 10 mg/kg’ to ‘limit of quantification of 10 μg/kg’.
Page 65:
'Cypermethrin'
line 1, replace 'Acceptable daily intake: 0–50 mg/kg bw' to 'Acceptable daily intake: 0–50 μg/kg bw'.
Footnote a, line 5: replace 'a-cypermethrin' by 'α-cypermethrin'.

Page 66:
'α-Cypermethrin', line 1: replace 'Acceptable daily intake: 0–20 mg/kg bw' to 'Acceptable daily intake: 0–20 μg/kg bw'.
'Phoxim', line 1, replace 'Acceptable daily intake: 0–4 mg/kg bw' to 'Acceptable daily intake: 0–4 μg/kg bw'.
Production aid, Melengestrol acetate', line 1, replace 'Acceptable daily intake: 0–0.03 mg/kg bw' to 'Acceptable daily intake: 0–0.03 μg/kg bw'.