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PAPER

41/15

Residues of some veterinary drugs in animals and foods

Carbadox
Deltamethrin
Dicyclanil
Flumequine
Imidocarb dipropionate
Neomycin

41/15

Residues of some veterinary drugs in animals and foods

FAO

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Monographs prepared by the
sixtieth meeting of the
Joint FAO/WHO Expert Committee
on Food Additives

Geneva, Switzerland, 6–12 February 2003

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Geneva, 6–12 February 2003

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ABBREVIATIONS

1R	Gifford and Dunsire (1994)
2R	McLean and Dunsire (1996)
3R	Anderson and Speirs (1998)
4R	Thanei (1996a)
5R	Phillips (1996)
6R	Loeffler (1998)
ADI	Acceptable Daily Intake
AOAC	AOAC International (Association of Analytical Communities)
APCI	Atmospheric pressure chemical ionisation
AUC	area under the curve
bw	Body weight
CAS	Chemical Abstracts Service
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CCRVDF	
CGA 183893	5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine; dicyclanil
CGA 297107	2-4,6-triamino-pyrimidine-5-carbonitrile
CV	Coefficient of variation
dpm	decays per minute
ECD	Electron Capture Detector
EDTA	ethylenediaminetetraacetic acid
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	US Food and Drug Admistration
GC	Gas chromatography
GEMS Food	Global Environment Monitoring System/ Food Contamination Monitoring and Assessment Programme
GLP	Good Laboratory Practice
HPLC	High pressure liquid chromatography
IEC	Ion exchange chromatography
IEDI	International estimated daily intake
IESTI	International estimate of short-term intake
IR	Infrared
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC	liquid chromatography
LCL	lowest calibrated level
LOD	Limit of detection
LOQ	Limit of quantitation
LSC	Liquid scintillation counting
MB	microbiological method
MET 1U	N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide
MET 2U	5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine; dicyclanil
MET 3U	2-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-3-hydroxy-propionic acid
MET 4U	2-4,6-triamino-pyrimidine-5-carbonitrile
MET 5U	3-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-propionic acid
MIC	Minimal Inhibitory Concentration
MRL	Maximum Residue Limit
MS	Mass spectrometry
N	Negative
NADA	New animal drug application
ND	Not detected
NI	Not investigated
NICI	negative ion chemical ionization
NMR	nuclear magnetic resonance
NQ	Not quantifiable
P	Positive
PES	Post-extracted solids
QC	Quality control
QCA	quinoxaline-2-carboxylic acid
RfD	Aacute dietary reference dose
SC	Subcutaneous (injection)
SD	Standard deviation
SPE	Solid Phase Extraction
Std.er.	Standard error
STMR	Supervised trial median residue values
TLC	Thin layer chromatography
TMDI	Theoretical maximum daily intake
TRR	Total radioactive residue
TRS	Technical Report Series
U	uncertain
USP	United States Pharmacopoeia

INTRODUCTION

The monographs on the residues of, or statements on, the veterinary drugs contained in this volume were prepared by the 60th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 6 – 12 February 2003. The Committee has evaluated veterinary drugs at previous meetings, including the 12th[1], 26th[2], 27th[3], 32nd[4], 34th[5], 36th[6], 38th[7], 40th[8], 42nd[9], 43rd[10], 45th[11], 47th[12], 48th[13], 50th[14], 52nd[15], 54th[19] , and 58th[20] meeting.

Background

In response to a growing concern about mass-medication of food producing animals and the potential implications for human health and international trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984[16]. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA[17]. In response to these recommendations, the 32nd JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, fourteen meetings of JECFA were dedicated exclusively to evaluation of veterinary drugs.

60th Meeting of JECFA

The present volume contains monographs of the residue data on six of the eight compounds on the agenda. The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the 60th meeting is included in Annex 1. A summary of the recommendations on compounds on the agenda and further information required is included in Annex 2.

The monographs of this volume must be considered in context of the full report of the meeting, which will be published in the WHO Technical Report Series.

Acknowledgements

The assistance of the FAO experts and members of the Secretariat who prepared these monographs is gratefully acknowledged. The Secretariat wishes to thank Dr Il Ho Cho for support in the editorial work of this publication.

On-line edition of ***Residues of some veterinary drugs in animals and foods***

The monographs and statements that have been publishedin the FAO Food and Nutrition Paper 41 (fifteen volumes since 1988) are now available for online at www.fao.org/es/esn/jecfa/archive_en.stm. The search interface is available in five languages (Arabic, Chinese, English, French, Spanish) and allows to search for compounds, functional classes, ADI and MRL status. For each veterinary drug ever assessed by the Committee an excerpt is available that summarizes the opinion of JECFA with respect to ADI and/or MRL.

Contact & Feedback

More information on the work of the Committee is available from the FAO homepage of JECFA at www.fao.org/es/ESN/jecfa/index_en.stm. Readers are invited to address comments and questions on this publication and other topics related to the work of JECFA to:

jecfa@fao.org

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CARBADOX

First draft prepared by

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Dieter Arnold, Berlin, Germany

ADDENDUM

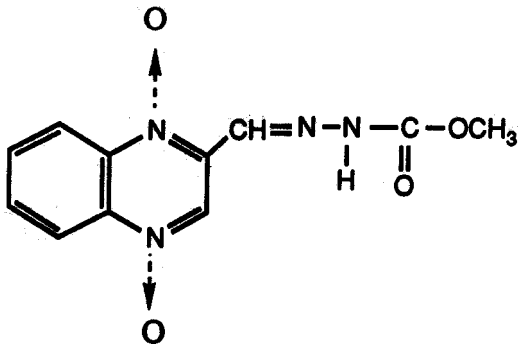
To the carbadox monograph prepared by the 36th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/3, Rome 1991

IDENTITY

Chemical name: Methyl-3-(2-quinoxanyl-methylene) carbazate-N1,N4-dioxide.

Common trade names: Mecadox; Fortigro;GS-6244; Nutriton; Getroxel.

Structural formula:



Molecular formula: $C_{11}H_{10}N_4O_4$

Molecular weight: 262.2

INTRODUCTION

Carbadox was first reviewed by the Committee at its thirty-sixth meeting in 1990 (WHO 1990). In reaching its decision on MRLs for carbadox, the Committee took the following factors into consideration:

- Because of the genotoxic and carcinogenic nature of carbadox and its metabolite desoxy-carbadox, the Committee was not able to establish an ADI.
- Carbadox and desoxy-carbadox can only be detected (<5µg/kg) in tissues for the first 72 hours after treatment, their levels at 28 days withdrawal are negligible.
- More than 90% of total residues in tissues were bound residues and unextractable at 28 days withdrawal.
- With current analytical procedures, quinoxaline-2-carboxylic acid (QCA) is the only carbadox metabolite that can be identified in liver from pigs treated according to good practice in the use of veterinary drugs.
- Bound residues in swine liver at 28 days after treatment would not represent a risk for consumers.
- A 28 day withdrawal time was suitable for residues not to present any risk to consumers.
- Quinoxaline-2-carboxylic acid extracted by alkaline hydrolysis was less than 30 µg/kg after 28 days withdrawal.

- Practical analytical methods are available for measuring quinoxaline-2-carboxylic acid to 30 µg/kg in liver and to 5 µg/kg in muscle.

On the basis of data from studies on the toxicity of quinoxaline-2-carboxylic acid, and on the metabolism and depletion of carbadox, and the nature of the compounds released from the bound residues, the Committee concluded that residues resulting from the use of carbadox in pigs were acceptable and recommended MRLs of 30 µg/kg in liver and 5 µg/kg in muscle of pigs, based on the levels of, and expressed as, quinoxaline-2-carboxylic acid.

The 13th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) requested that carbadox be given priority for review by the Joint FAO/WHO 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 Joint Secretariat to JECFA requested requested the following information:

- - All relevant toxicology and residue data, including analytical methods for detecting the parent drug and metabolites in tissues of pigs that have been generated sine the previous evaluation by JECFA.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

General

Carbadox (GS-6244) is an antimicrobial drug used in the feed of swine for growth promotion, improved feed efficiency, increased rate of weight gain, and to control swine dysentery and bacterial swine enteritis. The commercial product is intended for use in starter and/or grower rations but not in finisher rations.

Dosage

It is usually administered orally in finished feed at 55 mg/kg (50g/ton). (FDA, 1998)

Previous studies of the metabolism of carbadox

The metabolism of carbadox has been studied in rats, monkeys and pigs using [^{14}C] carbadox. labelled in either the *phenyl* ring or the *carbonyl* group of the side-chain. The metabolism of carbadox was characterized by the rapid reduction of the N-oxide groups to give desoxycarbadox, the cleavage of the methyl carbazate side-chain to give the carboxaldehyde and the corresponding carboxylic acid, and the liberation of respired CO_2. The detectable residues in tissues, up to 24 hours after drug withdrawal, were carbadox, desoxycarbadox, quinoxaline-1,4-di-N-oxide-2-carboxaldehyde, and quinoxaline-2-carboxylic acid (QCA). QCA was the only residue in liver detected 24 hours or longer after dose. The metabolism of ring labelled carbadox is summarized in Figure 1 (Pfizer, 1989a).

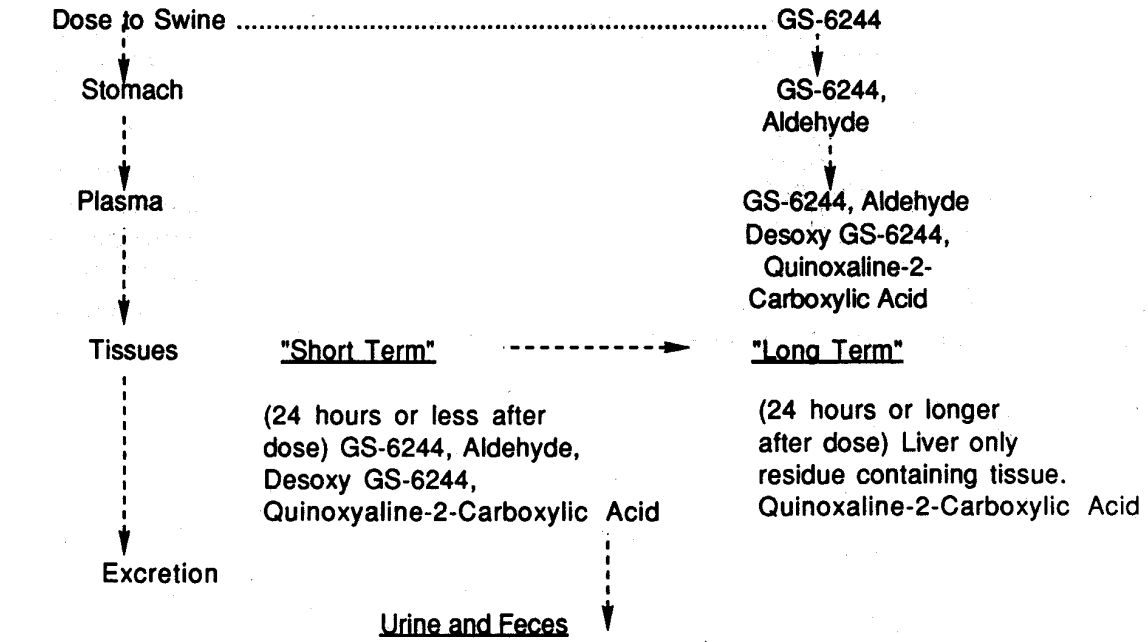
Studies with *carbonyl* labelled carbadox have demonstrated that methylcarbazate is generated. Most of the methylcarbazate is enzymatically hydrolyzed to yield CO_2. Radioactivity in liver decreased with a half-life of two days, and five days after dosing corresponded to 0.12 mg/kg methylcarbazate equivalent that was shown to consist in part of amino acids which were labeled by incorporation of $^{14}CO_2$. The enzymatic hydrolysis of methylcarbazate implies but does not prove the formation of hydrazine. Studies with appropriately labeled drug can measured the residues in tissues arising from methylcarbazate or from quinoxaline derivatives, but no radiotracer method can demonstrate the absence of hydrazine. However, hydrazine was a minor metabolite and would be expected to be present only for a short time before undergoing further metabolism since several enzymatic processes are known to destroy hydrazine. In plasma, free hydrazine was not detected by an assay with a limit of detection of 0.1 mg/kg. The metabolism of carbonyl labeled carbadox is summarized in Figure 2 (Pfizer, 1989a).

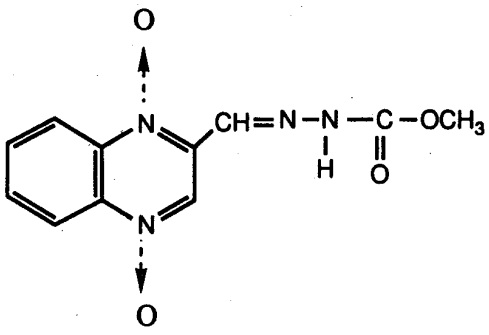
Peak radioactivity concentrations in plasma were observed at approximately 3 hours after dosing, indicating good oral absorption. Carbadox, its aldehyde, desoxy-carbadox and QCA were present in plasma within hours after drug administration, but had disappeared 24 hours later.

Two thirds of the dose was rapidly eliminated with the urine within 24 hours, the remaining with the feces. The major urinary metabolite was shown to be the QCA, which was also excreted in conjugated form. No N-oxides were found in urine. Feces contained some QCA and no unchanged carbadox.

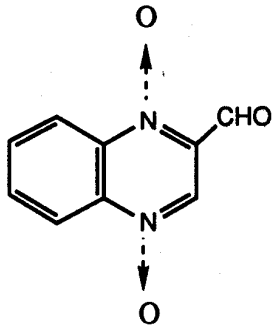
Figure 1

Summary of Ring Labeled Carbadox (GS-6244) Metabolism in Swine

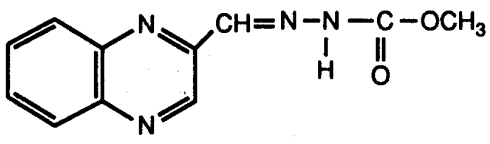




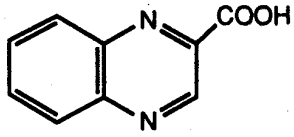
GS-6244



GS-6244 Aldehyde
(Quinoxaline-1, 4-di-N-Oxide-2-Carboxaldehyde)



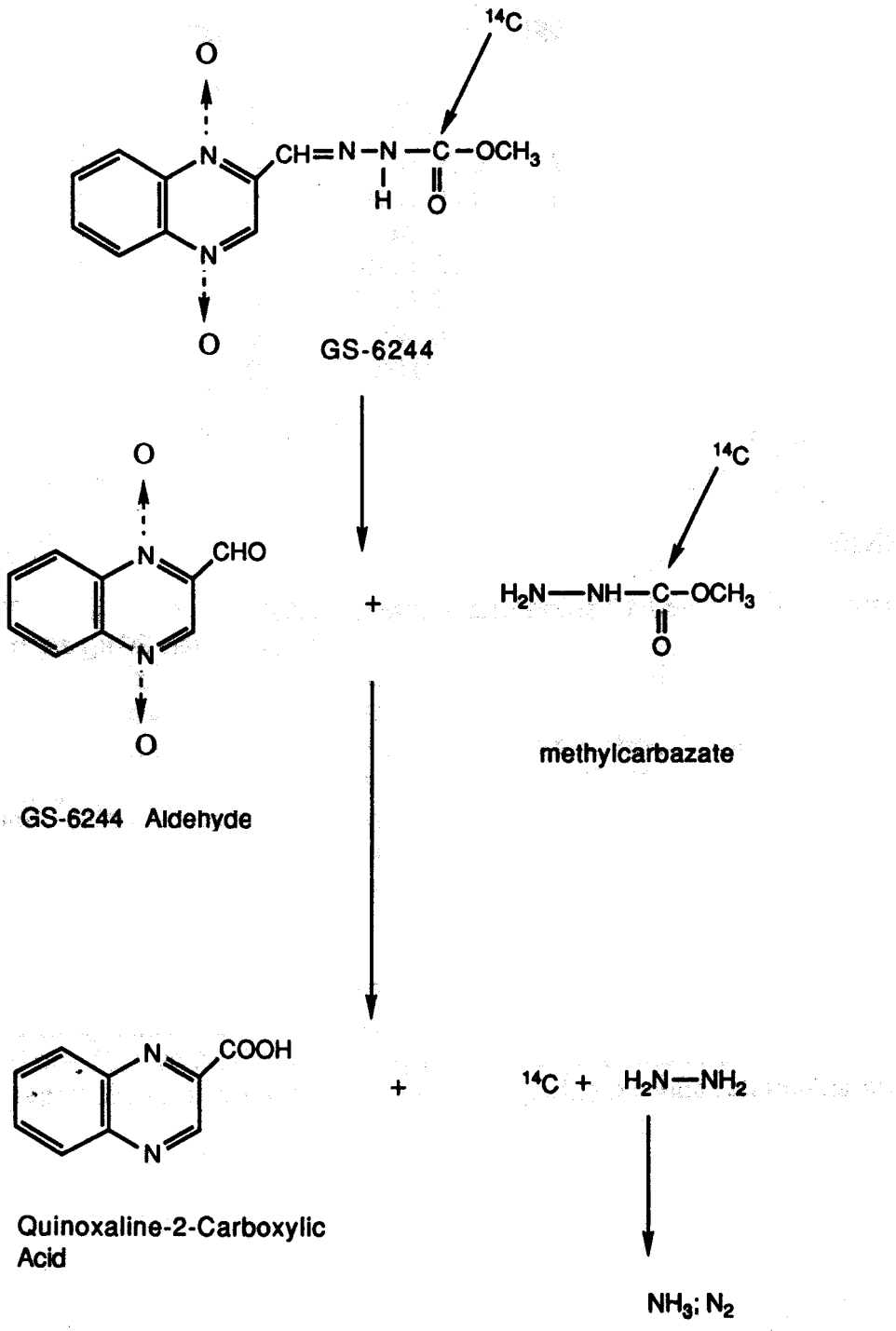
Desoxy GS-6244
(Methyl 3-(2-Quinoxalinylmethylene Carbazate)



Quinoxaline-2-Carboxylic Acid

Figure 2

Summary of ^{14}C-Carbonyl Labeled Carbadox Metabolism in Swine



Residue data

A depletion study of carbadox and desoxycarbadox (MacIntosh et al., 1985) was made in young pigs fed with carbadox-containing rations (55 mg/kg) for one week using a liquid chromatographic method (LOD 2 µg/kg). The presence of carbadox and desoxycarbadox was reported in swine tissues until 72 hours post dose using a single animal at each time point. Results are shown in table 1.

Table 1. Residues (µg/kg) of carbadox and desoxycarbadox in tissues of treated pigs (55 mg/kg, 7 days)

Withdrawal period	Carbadox			Desoxycarbadox		
	Liver	Muscle	Kidney	Liver	Muscle	Kidney
24 hours	<2	19	<2	125	17	186
48 hours	<2	<2	<2	17	9	34
72 hours	<2	<2	<2	<2	<2	<2

This work predicted the possibility of the presence of carbadox and desoxycarbadox in tissues from a pig treated with carbadox that had not been withdrawn.

A pivotal study (Pfizer, 1989c) was conducted in which 10 swine were given unrestricted access to feed containing 55 mg/kg [^{14}C] carbadox (uniformly labelled in the phenyl ring, specific activity of 8.4 µCi/g) for 5 consecutive days and killed at 30, 45 and 70 days, resp., after treatment. Concentration of total residues was measured in tissues (LOD = 1µg/kg).

Table 2. Concentration (µg/kg of carbadox equivalents) of total residues in tissues of treated pigs (55 mg/kg, 5 days)[a]

Withdrawal time (days)	Liver	Kidney	Muscle	Fat
30	74 (50-117)	15 (10-21)	5 (3-6)	2 (1-3)
45	20 (17-21)	5 (4-6)	3 (2-4)	1
70	13 (13-14)	4 (3-4)	2 (2-3)	<1

[a] Numbers in parentheses refer to the range of values obtained.

In another study (Pfizer, 1989d), which was similarly designed, however, in which feed consumption was lower, the tissues were assayed for extractable and bound radioactivity, following sequential extraction with methanol, acetone and n-hexane. The results showed that more than 90% of the total residues in tissues at 30 and 45 days withdrawal time were non-extractable.

The concentration of carbadox and desoxycarbadox in the tissues declined rapidly, and were less than 5µg/kg after 3 days. Extremely low levels of unidentified metabolites remained in the liver at withdrawal periods longer than 7 days. These residues were partially released and converted to quinoxaline-2-carboxylic acid by alkaline digestion of the liver.

The liver tissue was assayed for QCA (as measured by methyl quinoxaline-2-carboxylate) by a method involving alkaline digestion, thin-layer chromatography, gas-1iquid chromatography, and reverse-isotope dilution. The concentration of the residue was 18.9 µg/kg at 30 days withdrawal time, decreasing to 5.5 µg/kg at 45 days, and 1.3 µg/kg at 70 days, representing 24.4 %, 27.5 % and 9.9 % of the total residues respectively.

A study aimed at characterizing the bound residue was conducted using [^{14}C]carbadox (labelled in the phenyl ring). Liver samples obtained after one week following withdrawal of the medicated feed were extracted with enzymes under acidic, neutral, and alkaline conditions. However, no more than 19% of the radioactivity was extractable and no major metabolites could be identified.

Bioavailability studies of the bound residues by *in vivo* methods were not considered feasible because of the low level of residues in the liver tissues at withdrawal periods of four weeks.

Eighteen swine with an average body weight of 25 pounds were fed a ration containing carbadox at 55 mg/kg (50 g/ton) continuously for 47 days until they reached 103 pounds body weight. At this point (zero withdrawal) three swine were sacrificed followed by an additional three swine on each of the days 7, 14, 21, 28, and 35 days of drug withdrawal. The method had a limit of quantification of 30 µg/kg. Residues were below the MRL in liver at 28 days withdrawal. In muscle, they were below the LOQ at all times analyzed. Results are summarized in table 3.

Table 3. QCA levels (µg/kg) in tissues of swine following 47 days of feeding with carbadox (55 mg/kg)

Withdrawal time (days)	Liver	Kidney	Muscle
0	345 (293-400)	210 (142-299)	<LOQ
7	168 (153-186)	<LOQ	<LOQ
14	82.6 (70-102)	<LOQ	<LOQ
21	45.6 (40-49)	<LOQ	<LOQ
28	<LOQ	<LOQ	<LOQ
35	<LOQ	<LOQ	<LOQ

Numbers in parentheses refer to the range of values obtained.

New information about residues of carbadox

Two new studies were provided, supplying new information on depletion of residues of carbadox from 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.

Study carried out to determine a withdrawal time on the basis of the MRL for QCA: *Depletion of QCA residue in liver by growing swine after consumption of carbadox and oxytetracycline in combination*

The combination of carbadox and oxytetracyc1ine is indicated for increased rate of weight gain, improved feed efficiency, treatment of bacterial enteritis caused by *Escherichia coli* and *Salmonella cholerae suis* sensitive to oxytetracyc1ine and treatment of bacterial pneumonia caused by *Pasteurella multocida* sensitive to oxytetracyc1ine. In order to gain regulatory approval to use both products in a single feed, it was necessary to demonstrate that oxytetracycline use would not increase the tissue concentration of the carbadox marker residue above its tolerance limit.

Pigs were fed a diet of 27.5 mg/kg (25g/ton) carbadox in combination with 880 mg/kg oxytetracycline (Heird and Spires, 2002). Carbadox was incorporated into the feed of the other seven pens for 28 days and oxytetracycline was added in combination with carbadox for the last 14 days before the withdrawal period. Animals used in the study had an average body weight of approximately 63 pounds when they first began receiving a diet containing carbadox. The average body weight was 119 pounds when withdrawal began and increased to 203 pounds at the time of slaughter of the last group of animals.

Five animals were sacrificed at each of 7 different time points. The livers were analyzed for QCA (Lynch and Bartolucci, 1982) in order to propose a regulatory withdrawal time on the basis of the MRL recommended by the 36th JECFA. All results were corrected for recoveries. Results are presented in Table 4.

Table 4 . QCA depletion (µg/kg) in liver of swine treated with feed containing 27.5 mg/kg of carbadox

Withdrawal time (days)	No. Observations*	Mean	SD
0	5	133	74
7	5	41	9.3
14	5	28.7	11.8
21	5	6.7	3.1
28	4	3.1	0.095
35	1	2.3**	-
42	2	2.15	0.071

* QCA was below the LOD (2 µg/kg) in one of five animals sacrificed at 28 days, in four of the five animals sacrificed at 35 days, and in three of the five animals sacrificed at 42 days after withdrawal of carbadox. LOQ = 5 µg/kg.

** This value constitutes the detectable observation in only one of the five animals

Based on these results, the QCA depletion curve was fitted using data for groups of pigs slaughtered after 7, 14, 21 and 28 days of withdrawal. Data from 35 and 42 days after last dose were not used in the statistical analysis. A linear model of ln [QCA] vs. time was highly significant ($P < 0.0001$) and the lack of fit test departure from linearity was non-significant ($P = 0.0553$).

The statistical methods used were in agreement with methods proposed by the Center for Veterinary Medicine Guideline (FDA, 1994). Based upon the fitted linear model, and using a statistical tolerance limit for the 99 th percentile of the population with 95 % confidence, a withdrawal period of 22 days was calculated to ensure that the liver QCA concentration, calculated as 13.1µg/kg was below the 30 µg/kg tolerance limit

Short term to describe the depletion of residues during the first 15 days after withdrawal of the drug

A short-term withdrawal residue study was conducted using pigs (13 barrow and 13 gilts) with a range of body weights of 100-125 pounds. The animals were fed a diet containing the maximum approved concentration of 50 g/ton of feed (55mg/kg) for 14 days. Three animals were sacrificed at each of the following withdrawal times: 0, 3, 6, 9, 12, 24 hours, 2, 4, 7, 10, and 15 days. The tissues collected were: muscle, liver, kidney, skin, and fat. Medicated diets were prepared by mixing carbadox into the basal diet and carbadox concentrations were analyzed by validated HPLC methods to ensure adequate homogeneity.

Carbadox, desoxycarbadox and QCA were determined directly in untreated samples of the tissues, in whole tissue samples after incubation with USP simulated gastric fluid (pepsin), in whole tissue samples after incubation with USP simulated intestinal fluid (pancreatin), and in the supernatant of samples after treatment with simulated digestive fluids. QCA was determined by the regulatory GC-ECD method. Residues of carbadox and desoxycarbadox were determined quantitatively by liquid chromatography with tandem mass spectroscopy (LC/ACPI-MS/MS) after extraction with acetonitrile. After enzymatic treatment of the samples, residues of carbadox, desoxycarbadox and QCA were determined by LC/ACPI-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.

The results of this residue depletion study are shown in figures 5 and 6 and in tables 5, 6, 7 and 8. QCA was the main residue in liver followed by desoxycarbadox. Pretreatment of the samples with digestive enzymes increased significantly the amounts of residues found in the tissue. In particular the levels of carcinogenic desoxycarbadox increased by a factor of over 4 when the samples were treated with pancreatin prior to extraction and analysis.

QCA was not detected in muscle, except in two samples taken at early withdrawal times. There was a steep decrease in the concentrations of carbadox and desoxycarbadox in muscle during the first few hours following withdrawal of the medicated feed. The only residue remaining quantifiable until the end of the study was desoxycarbadox.

QCA was not detected or quantified in skin or fat. In skin tissue carbadox residue values are higher than in liver or muscle, being desoxy-carbadox values lower than in those tissues. Levels of carcinogenic residues were increased by pretreatment of samples with enzymes in some cases, but only one sample per time point was analyzed. Carbadox

Figure 5: Depletion of residues of carbadox in liver of swine treated with carbadox at 55 mg/kg for 14 days

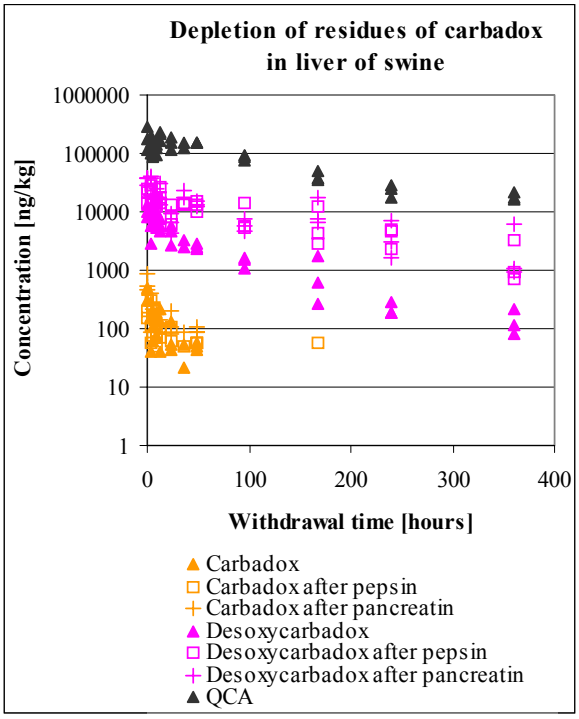
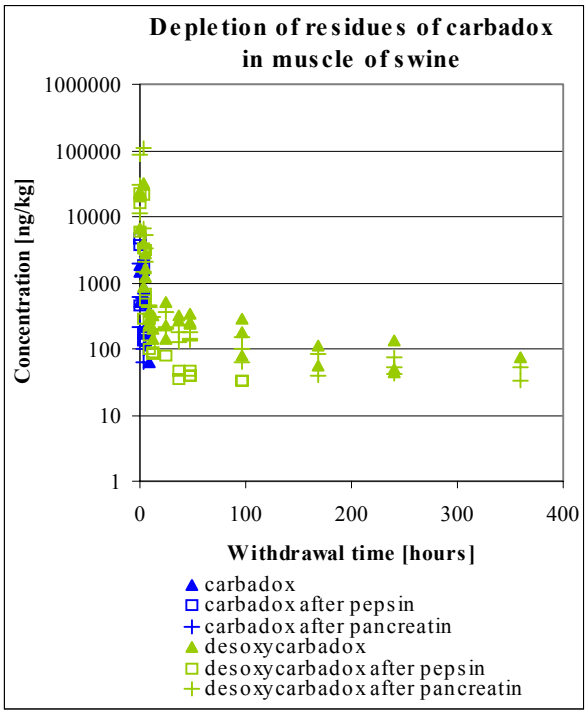


Figure 6: Depletion of residues of carbadox in muscle of swine treated with carbadox at 55 mg/kg for 14 days



was not detectable at 10 days withdrawal in all samples and desoxy-carbadox was the only residue quantifiable at 15 days withdrawal.

In fat tissue, enzyme treatment of samples increased carbadox and desoxy-carbadox concentrations only in a few samples during the first hours withdrawal time (only one sample analyzed per time point). Carbadox tissue values were very variable with time, being desoxy-carbadox levels lower than those in liver or muscle. Carbadox and desoxycarbadox were non quantifiable between 7 and 10 days withdrawal.

A single sample of kidney at 0, 6, 12, 24, 48 and 96 hours withdrawal time was analyzed to measure carbadox, desoxy-carbadox and QCA. These samples corresponded to the animal liver values that were the highest at each time point. Carbadox and QCA were non quantifiable or were present in very low values after 0 hour withdrawal time. When compared to liver levels, desoxycarbadox values values showed a steep decrease during the first hours and are under 424 ng/kg at 96 hours withdrawal time.

Table 5: Carbadox, desoxy-carbadox and QCA in swine liver tissue, fluid extractions and supernatants

Withd. Time	Carbadox (ng/kg)					Desoxy-carbadox (ng/kg)					QCA (µg/kg)
	Tissue	Gastric fluid	Intestinal fluid	Gastric sup.	Intestinal sup.	Tissue	Gastric fluid	Intestinal fluid	Gastric supernatant	Intestinal supernatant	Tissue
0 hours	423 ± 82	173 ±23	621 ±163	150 ±30	590 ±5	10,506±2,151	20,030±4,308	34,666 ±4,437	4,306 ±59	11,800 ±1435	188 ±69
3 hours	119 ±56	153 ±102	244 ±127	99 ±26	305 ±187	5,510 ±2,151	21,500 ±5,880	32,066 ±9,816	3,102 ±1,714	12,106 ±5,256	152 ±75
6 hours	123 ±55	153 ±57	250 ±30	126 ±36	233 ±88	7,260 ±1,069	10,976 ±2,137	11,246 ±4,716	3,550 ±665	11,233 ±974	123 ±32
9 hours	139 ±47	68 ±13	137 ±23	53 (x)	307 ±49	7,750 ±1,805	14,553 ±1,500	19,466 ±3,350	3,586 ±1,052	16,866 ±2,347	119 ±20
12 hours	128 ±71	85 ±12	122 ±14	99 ±22	252 ±56	5,590 ±1,160	19,166 ±5,253	19,700 ±7,239	3,873 ±627	24,666 ±1,915	201 ±30
24 hours	77 ±40	82 ± 22	131 ±55	85 ±13	226 ±62	4,383 ±1,294	9,066 ±2,826	6,846 ±1,997	3,849 ±2,968	21,200 ±978	150 ±26
36 hours*	37 ±15	50 (x)	87 (x)	NQ, ND	154 ±50	2,970 ±420	13,600 ±3,000	20,700 ±2,900	740 ±202	1,705 ±235	141 ±14
48 hours	50 ±7	57.5±0.5*	93 ±8	NQ, ND	168 ±24	2,230 ±256	12,933 ±2,200	13,600 ±1,267	690 ±66	16,966 ±1,144	152 ±2
96 hours	NQ	NQ	NQ	NQ, ND	83 ±14	1,413 ±265	8,293 ±3,900	6,006 ±1,115	1359 ±980	8,156 ±2,642	86.0 ±7.8
7 days	NQ	56 (x)	NQ, ND	NQ, ND	NQ, ND	875 ±628	6,500 ±4,280	10,390 ±4,970	247 ±194	203 ±280	41.0 ±7.2
10 days	NQ	NQ	NQ, ND	NQ, ND	NQ, ND	219 ±48	4,067 ±1,218	3,930 ±2,244	82.0 ±10.2	667 ±198	23.0 ±4.5
15 days	NQ	NQ, ND	NQ, ND	NQ, ND	NQ, ND	138 ±56	1,613 ±1,131	2,693 ±2,387	79.3 ±28.7	210 ±101	18.3±2.0
Controls	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ, ND

Data expressed as the mean (n=3 ±SD). (x) only one detectable value ;* only two values
LOQ Carbadox = 50 ng/kg, Desoxycarbadox = 30 ng/kg, QCA = 15 ng/kg. QCA JECFA MRL = 30 µg/kg
ND= non detected, NQ = non quantificable, ND < NQ < LOQ.

Table 6 : Carbadox, desoxy-carbadox and QCA in swine muscle tissue, fluid extractions and supernatants

Withd. Time	Carbadox (ng/kg)					Desoxy-carbadox (ng/kg)					QCA
	Tissue	Gastric fluid	Intestinal fluid	Gastric sup.	Intestinal sup.	Tissue	Gastric fluid	Intestinal fluid	Gastric supernatant	Intestinal supernatan	Tissue
0 hours	1292±585	2961±1818	946 ±767	874±727*	153 ± 70*	16916 ± 7248	14751± 6635	42351±32063	6522 ±298*	21987±14302	18 (x)
3 hours	990 ± 369	732 ± 814	250 ±241	185 ±168	94 ± 40*	11989±13700	8245 ± 9188	38851±49176	3557 ±4471	1592 ± 18510	16 (x)
6 hours	491 ± 228	147 ± 17*	119 (x)	108 (x)	NQ	1881 ± 662	1452 ± 1210	3591 ± 1308	472 ± 132	3376 ± 756	ND
9 hours	64 (x)	NQ, ND	NQ, ND	NQ, ND	NQ	299 ± 55	182 ± 78	445 ± 14	234 ± 38	2283 ± 24	ND
12 hours	ND	NQ, ND	NQ, ND	NQ, ND	NQ	211 ± 72	86.5 ± 2.5	230 ± 47	136 ± 30	141 ± 37	ND
24 hours	NQ, ND	NQ, ND	NQ, ND	NQ, ND	NQ	295 ± 156	77 (x)	258 ± 69	132 ± 50	104 ± 30	ND
36 hours	NQ, ND	ND	NQ, ND	ND	NQ	295 ± 32*	42 ± 6*	152 ± 23*	ND	38 ± 2*	ND
48 hours	ND	ND	NQ, ND	ND	111 (x)	280 ± 44	41 ± 3	153 ± 20	30 (x)	39 ± 6	15 (x)
96 hours	ND	ND	NQ, ND	ND	ND	182 ± 85	33.5 ± 0.5*	103 ± 34	NQ	31 (x)	ND
7 days	ND	ND	NQ, ND	ND	NQ, ND	83 ± 28*	NQ	62.5 ± 22.5*	NQ	NQ	ND
10 days	ND	ND	NQ, ND	ND	ND	76 ± 41	NQ	56.0 ± 13.0	NQ	NQ	ND
15 days	ND	ND	NQ, ND	ND	ND	74 (x)	NQ	43.0 ± 9.0*	NQ	NQ	ND
Controls	NQ, ND	NQ, ND	NQ, ND	ND, NQ	ND, NQ	ND, NQ	ND,NQ	ND,NQ	NQ, ND	NQ, ND	ND,NQ

Data expressed as the mean (n=3 ± SD) . (x) only one detectable value ; * only two values
LOQ Carbadox = 50 ng/kg, Desoxycarbadox = 30 ng/kg, QCA = 15 ng/kg. QCA JECFA MRL = 5 µg/kg
ND= non detected, NQ = non quantificable, ND < NQ < LOQ.

Table 7: Carbadox, Desoxy-carbadox and QCA in Swine Skin Tissue, Fluid Extractions and Supernatants

Withd. Time	Carbadox (ng/kg)					Desoxy-carbadox (ng/kg)					QCA
	Tissue [a]	Gastric fluid [b]	Intestinal fluid [b]	Gastric sup.[b]	Intestinal sup.[b]	Tissue [a]	Gastric fluid [b]	Intestinal fluid [b]	Gastric sup.[b]	Intestinal sup.[b]	Tissue [b]
0 hours	1843 ±1034	1290	382	1520	174	6560 ±2342	143	15300	127	10800	NQ
3 hours	1062 ± 640	647 ± 37	186 ±26	1320±409	303 ±102	6476 ±7594	111 ±58	11765 ±5935	57 ±20	8235 ±4465	NQ
6 hours	782 ± 277	339	150	227	167	1800 ± 652	73	2560	41	933	NQ
9 hours	1167 ± 456	95	853	736	147	1224 ±378	3440	35	43	2270	ND
12 hours	649 ± 277	210	816	756	81	538 ± 83	3420	40	37	2570	ND
24 hours	773 ± 446	172	625	907	166	732 ± 513	5540	107	93	3700	ND
36 hours	585 ± 348*	107	810	690	96	374 ± 11	2900	NQ	43	2160	ND
48 hours	145 ± 38	75	197	185	73	223 ± 101	931	39	42	613	ND
96 hours	98 ± 29*	78	610	67	53	185 ± 86	312	NQ	NQ	222	ND
7 days	NQ,ND	NQ	76	NQ	NQ	83 ± 34	253	NQ	NQ	216	ND
10 days	ND	NQ	NQ	NQ	NQ	77 ± 28	144	NQ	45	108	ND
15 days	NQ,ND	NQ	NQ	ND	NQ	58 ± 8	100	NQ	NQ	94	ND
Controls	NQ, ND	ND	NQ, ND	ND,NQ	ND,NQ	ND,NQ	ND,NQ	ND,NQ	NQ, ND	NQ, ND	ND,NQ

[a] Data expressed as the mean (n=3 ± SD) ; [b]only one data per time point, 36 hours two values.
(x) only one detectable value ; * only two detectable values
LOQ Carbadox = 50 ng/kg, Desoxycarbadox = 30 ng/kg, QCA = 15 µg/kg.
ND= non detected, NQ = non quantificable, ND < NQ < LOQ.

Table 8: Carbadox, Desoxy-carbadox and QCA in Swine Fat Tissue, Fluid Extractions and Supernatants

Withd. Time	Carbadox (ng/kg)					Desoxy-carbadox (ng/kg)					QCA
	Tissue [a]	Gastric fluid [b]	Intestinal fluid [b]	Gastric sup.[b]	Intestinal sup.[b]	Tissue [a]	Gastric fluid [b]	Intestinal fluid [b]	Gastric sup.[b]	Intestinal sup.[b]	Tissue [b]
0 hours	444 ± 371	NQ	NQ	86	NQ	7710 ±3790	102	9210	42	6120	ND
3 hours	134 ± 77	NQ	NQ	NQ	61	5935± 6231	83	7780	72	10400	NQ
6 hours	526 (x)	NQ	NQ	NQ	80	1131± 538	313	NQ	NQ	257	ND
9 hours	456 ± 214	NQ	NQ	NQ	NQ	336 ± 81	NQ	110		118	ND
12 hours	323 ± 78	NQ	NQ	66	114	206 ± 32	NQ	70	NQ	71	ND
24 hours	1235 ± 703	NQ	NQ	137	113	159 ± 20	NQ	61	NQ	56	ND
36 hours	609 ± 65	NQ	NQ	NQ	90	93 ± 14	NQ	45	NQ	73	ND
48 hours	663 ± 319	ND	NQ	135	58	106 ± 68	NQ	41	NQ	39	ND
96 hours	353 ± 157	NQ	NQ	NQ	NQ	33 ± 2	NQ	33	NQ	NQ	ND
7 days	NQ	NQ	NQ	NQ	NQ	NQ	NQ	32	NQ	NQ	ND
10 days	NQ,ND	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	ND
15 days	NQ	NQ	NQ	NQ	NQ	NQ	ND	NQ	NQ	NQ	ND
Controls	NQ, ND	ND	NQ, ND	ND,NQ	ND,NQ	ND,NQ	ND,NQ	ND,NQ	NQ, ND	NQ, ND	ND,NQ

[a] Data expressed as the mean (n=3 ± SD) ; [b]only one data per time point, 36 hours two values.
(x) only one detectable value ; * only two detectable values
LOQ Carbadox = 50 ng/kg, Desoxycarbadox = 30 ng/kg, QCA = 15 µg/kg.
ND= non detected, NQ = non quantificable, ND < NQ < LOQ

Other relevant residue studies

US-FDA Supplement Evaluation of Carbadox (NADA 041-061) providing information for establishment of a 42 day slaughter withdrawal period for carbadox in swine tissues.

The Food and Drug Administration of the United States of America has published a residue study on which the legally established withdrawal time for carbadox was based. Thirty-four crossbred pigs (17 gilts and 17 barrows) were given feed containing 55 mg/kgcarbadox. Pigs were killed at 14, 21, 28, 35, 42 and 49 days post dose. Muscle and liver were collected from each animal for QCA residue analysis. All of the tissue samples were analyzed in triplicate. Residues of QCA were determined using the regulatory GC-EC method for QCA (Lynch and Bartolucci, 1982) described above. The method has a limit of quantification of 5 µg/kg. Results are shown in Table 9.

Table 9. Residues of QCA (µg/kg) in tissues of swine fed carbadox at 55 mg/kg for 28 days.

Withdrawal time (days)	No. of Animals	QCA concentration (µg/kg)	
		Liver	Muscle
14	5	51.93 ± 15.14	< LOQ*
21	5	29.09 ± 8.20	< LOQ
28	5	17.72 ± 4.72	< LOQ
35	5	11.23 ± 1.86	< LOQ
42	3	11.16 ± 2.13	< LOQ
49	1	10.90 ± 2.35	< LOQ

* LOQ liver, muscle = 5 µg/kg; LOD liver, muscle = 2 µg/kg

For the purpose of establishing a withdrawal period only the liver residues were used. The withdrawal period was based on a statistical analysis of the depletion data, using an upper tolerance limit containing 99 % of the population with a 95% confidence limit. Using the uncorrected residue data for liver from days 14 to 49, a withdrawal period of 39.34 days was calculated. Based on this data, a 42-day withdrawal time was established.

Comparative Depletion of Residues in liver: estimation of depletion times

The kinetics of the depletion of residues in tissues of treated swine were compared for the above three studies and two additional previous studies mentioned in the report of the 36th meeting of JECFA (Pfizer, 1989 and MacIntosh, 1985).

A number of studies were reviewed and – where possible - quantitatively evaluated using statistical methods, such as linear regression and calculation of statistical tolerance limits and depletion times. The following equation was generally used to describe the depletion of residues:

$$\log_{10} C_t = \log_{10} a + b \times t$$

where C_t is the predicted concentration of the residue at t, a given withdrawal time, a is the concentration extrapolated for zero withdrawal time and b is a rate constant describing the depletion. For all studies and all calculations t was expressed in hours. Upper limits of the 95% or 99% confidence interval for the upper one-sided tolerance limits on the 95th or 99th percentile were calculated for selected relevant studies as a function of the depletion time.

For QCA depletion in liver (Figure 7, table 10) the two newly submitted studies show striking similarities in the kinetic parameters which are reflected in similar calculated depletion times to MRL (11 and 10 days respectivelly), despite a twofold difference in the dose level.

On the other hand, there are significant differences in kinetic parameters between, on the one side, one of the studies evaluated at the 36th Meeting (Pfizer, 1989) and the study published by FDA and, on the other side, the new short term study, although all three studies had been performed at the same dose level. These differences have very significant influence on depletion times calculated from these data sets.

Table 10: Comparison of parameters of four different studies describing the depletion kinetics of QCA in liver of swine.

Study	Cabadox concentration mg/kg /days of feeding	Parameters of linear regression analysis			Depletion time to < MRL (days)	
		a[+]	b [hour^{-1}]	r	Based on averages	Based on tolerance limits
Pfizer 1989	55/47	5.5239	-0.0018	-0.9867	25	*
FDA 1998	55/28	4.8823	-0.0008	-0.9285	21	39.3
Phibro 2002, QCA study	27.5/42	5.1488	-0.0025	-0.9419	11	21.5
Phibro 2002, short term study	55/14	5.2027	-0.0029	-0.9292	10	16.8

[+] a is dimensionless (contents of the residues given in ppt)
* calculation not possible; individual data points are not published.

A comparison was also made between the depletion kinetics determined for desoxy-carbadox in liver and muscle of swine in two studies using the same dose level of 55 mg/kg of parent carbadox (short-term depletion, 14 days and MacIntosh, 1985, 7 days, second study only two data points). While the results obtained for residues in livers were quite similar, there was seemingly a large difference in the results obtained for muscle.

Table 11a summarizes the kinetic characteristics of the short term depletion of the relevant residues of carbadox in liver of swine treated with 55 mg/kg of Carbadox.

The values in Table 11a demonstrate that for the data sets relating to residues of desoxycarbadox the linear regression model fits satisfactorily to the data. This is less true for the carbadox residue data, in particular for the data obtained after treatment of the tissues with pepsin.

Treatment with digestive enzymes increases the recovered concentrations of both, carbadox and desoxy-carbadox. However, the effect is significant only with residues of desoxy-carbadox. Pancreatin is more effective than pepsin.

Figure 7: Comparison of the results of four different QCA depletion studies in liver of swine.

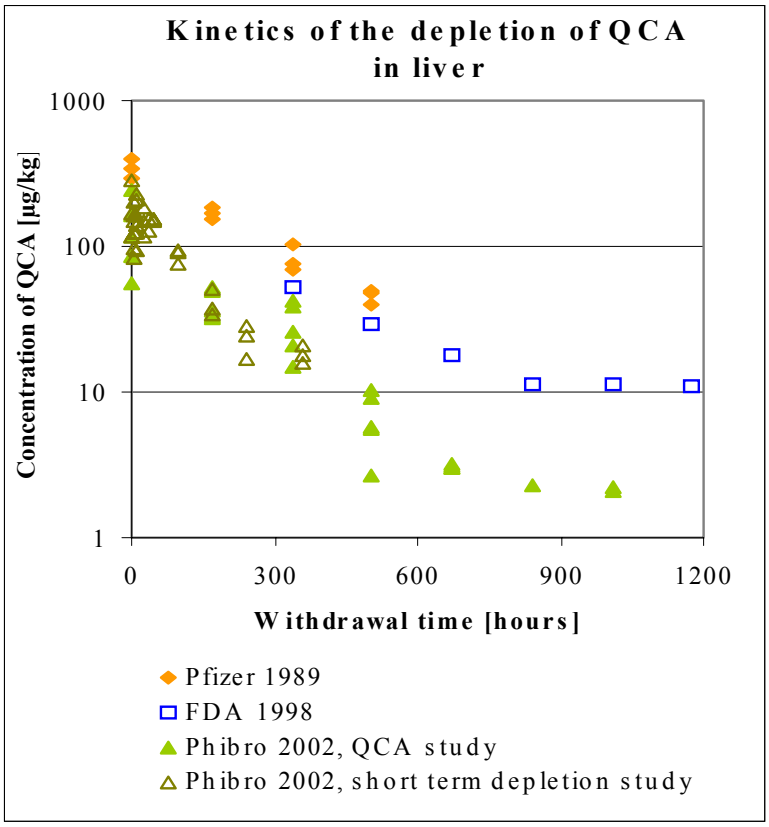


Table 11: Parameters of the kinetics of depletion of residues of carbadox in liver of swine - influence of the treatment of the tissue samples with digestive enzymes:

a) influence on the parameters obtained by linear regression

Residue	Enzymatic treatment	Parameters of the depletion kinetics			
		a[+]	b [hour^{-1}]	r	n
QCA	None	5.2027	-0.0029	-0.9292	35
Carbadox	None	2.2418	-0.0147	-0.6489	23
	Pepsin	2.0182	-0.0026	-0.4156	19
	Pancreatin	2.4231	-0.0120	-0.6519	22
Desoxy-carbadox	None	3.7876	-0.0053	-0.9464	35
	Pepsin	4.2042	-0.0029	-0.8707	35
	Pancreatin	4.2651	-0.0029	-0.7767	35

[+] a is dimensionless (contents of the residues given in ppt)

b) influence of the time required for the residues to deplete to certain limits/concentration

Residue	Regulatory Limits		Depletion times [days after withdrawal of medicated feed] on the basis of:						
	Type of Limit	Numerical value	averages	1-α 95	1-γ 95	1-α 99	1-γ 95	1-α 99	1-γ 99
QCA	MRL	30µg/kg	10.3	15.1		16.8		18.0	

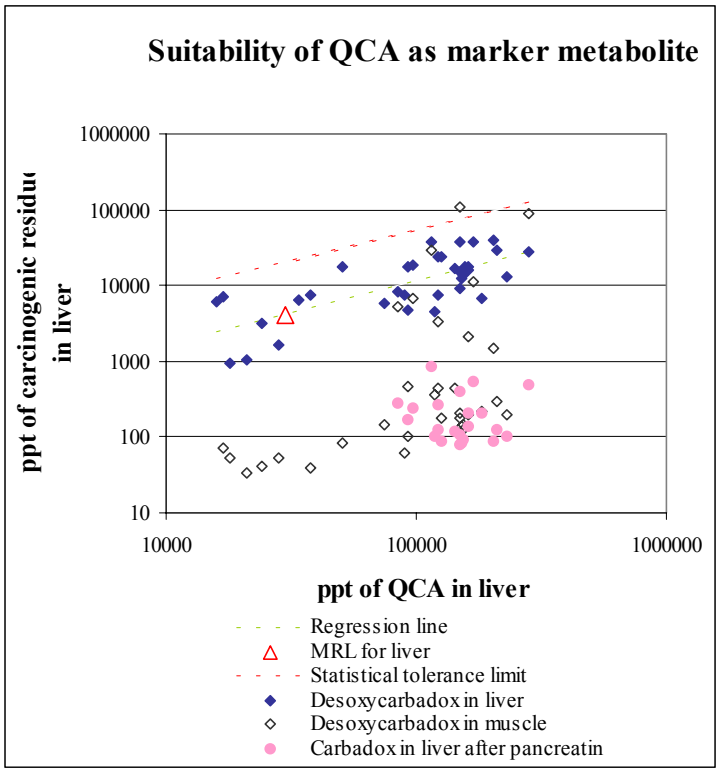
Table 11b provides estimates of required depletion times for QCA. Calculations have been performed on the basis of average concentrations of residues as well as on the basis of (statistical) tolerance limits. Three cases have been considered with respect to the percentiles and upper confidence limits chosen. (95% confidence interval on the 95th percentile, 99% confidence interval on the 95th percentile, 99% confidence interval on the 99th percentile).

Relationship between QCA and carcinogenic residues

The data of the short term depletion study were also used to establish a relationship between the concentrations of the proposed marker metabolite QCA in the target tissue liver and the carcinogenic residues carbadox and desoxy-carbadox in liver and muscle respectively. There is seemingly a linear relationship between the logarithms of the concentrations of QCA and desoxy-carbadox in liver (Figure 8). The relationship between QCA in liver and desoxycarbadox in muscle can only be used over a narrow range of concentrations.

At the MRL for QCA in liver, the average concentrations of the carcinogenic residue desoxy-carbadox in liver estimated by regression analysis were about 4 µg/kg.

Figure 8: Relationship between concentrations of QCA in liver and the concentrations of carcinogenic residues in liver and muscle



METHODS OF ANALYSIS

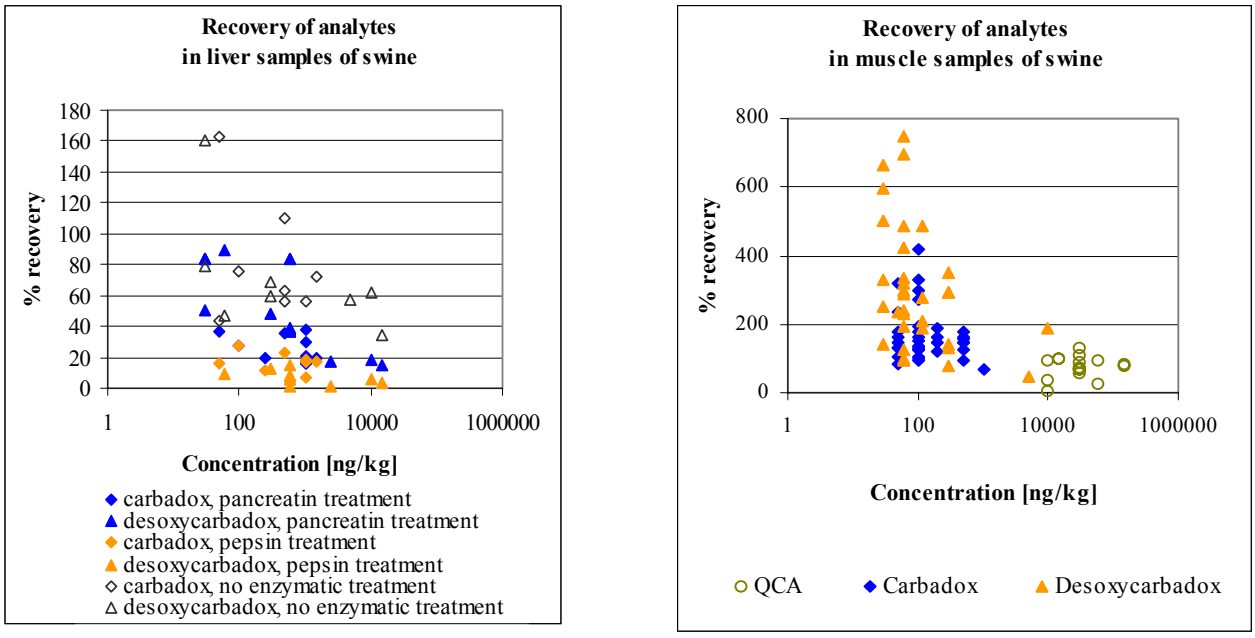
Two methods of determination were used in the three new residue studies considered by the Committee. The recognized regulatory method, based on GC-ECD and accepted by the Committee at its 36th meeting (Lynch and Bartolucci, 1982), was used in two studies to determine residues resulting from treatment 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 principle of the analytical method is as follows: a homogenized tissue sample is hydrolyzed in an alkaline medium, cooled and the hydrolysate acidified. QCA is extracted with ethylacetate. It is then re-extracted from the organic phase using a citric acid buffer. This extract is further cleaned on an ion-exchange column from which QCA is eluted with 14% methanol. After partitioning into chloroform, the solvent is evaporated and the residue is derivatised with n-propanol/sulfuric acid to form the propyl ester. The ester is extracted into toluene and measured using gas chromatography/electron capture detection. Average recoveries were 109.4 % at 10 µg/kg and 80.9% at 50 µg/kg.

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 and LOD of 0.002 mg/kg.

A new method based on liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry (LC/APCI-MS/MS) was developed for use in a recent study to measure the concentrations of carbadox, desoxycarbadox and QCA directly in tissues (Method EXM-049-037), or in the whole sample or supernatants obtained following treatment with simulated digestive fluids (pepsin or pancreatin) (Method EXM-049-037A). 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. This method was developed using liver as this represents the most difficult matrix to be used. Liver was preprocessed by grinding to a powder like consistency in the presence of dry ice. A 1g subsample was extracted with acetonitrile, centrifuged, partitioned in isooctane and evaporated to 2 ml. The final extract was analyzed by LC/MS/MS using a gradient (acetonitrile:water, 14 min) high performance liquid chromatograph system with a phenyl-hexyl column (150 x 4.6 mm x 3µm) and a variable volume injector capable of injecting 25 µl connected with a high sensitivity triple quadrupole Mass Spectrometer via an Atmospheric Pressure Electrospray Ionization Source (700ºC). Precursor to product ion transitions are monitored for each compound and plotted against an external standard curve (carbadox mass transition 263 ➔231, desoxy-carbadox mass transition 231 ➔ 199).(Retention time: carbadox 5.55 min, desoxy-carbadox 6.01 min)

When samples were incubated with digestive fluids the extraction procedure was different. In these cases sodium sulfate was added to the samples and the residues were three times extracted into ethyl acetate. In the case of pancreatin digestion formic acid was added prior to the third extraction with ethyl acetate. The ethyl acetate was evaporated and the residues were reconstituted into acetonitrile. When residues were determined in supernatants of the enzyme digestion the fluids were simply centrifuged and the clear supernatants were directly used for analysis.

Figure 9 (a, b) : Recoveries of analytes in swine tissues.



A linear response was found across the analytical range, with reported LOQs of 50 ng/kg for carbadox, 30 ng/kg for desoxycarbadox and 0.015 mg/kg for QCA. Analyte recoveries were generally variable with the liver samples obtained in this study and decreased to low levels when digestive enzymes were used prior to extraction (see figure 9a). Typically two fortified samples were analyzed under the same conditions with every set of samples in order to estimate recovery. No suitable internal standard was available for recovery correction. When muscle tissues were analyzed, the variability of the analyte recoveries

was even higher – depending on analyte and on sample treatment. The reasons for the unusually high "recoveries" of several hundred percent were not explained in the study. The results are shown in figure 9b.

Analysis of carbadox, desoxycarbadox and QCA after a 4 hour incubation with digestive enzymes showed that carbadox and desoxycarbadox were unstable in the samples treated with pepsin, but pancreatin has little effect on both compounds. In the samples incubated with liver, once again carbadox and desoxycarbadox were unstable in pepsin treated samples, but a decreased was noted for desoxycarbadox in pancreatin treated ones. QCA concentration was unaffected by the enzyme treatment. Results are shown in table 12 and table 13.

Table 12: Carbadox, Desoxycarbadox and QCA in Gastric and Intestinal Fluids: Recovery vs time

	Gastric Fluid: % Recovery			Intestinal Fluid: % Recovery		
Time	**Carbadox**	**Desoxy-C**	**QCA**	**Carbadox**	**Desoxy-C**	**QCA**
0 hours	18.4 ± 12.2	33.6 ± 10.1	98.4 ± 6.7	61.2 ± 5.1	94.1 ± 12.5	31.2 ± 13.7
4 hours	4.34 ± 3.15	0	118 ± 15	86.0 ± 5.9	77.6 ± 6.2	29.1 ± 12.6

Data expressed as the mean (n=12 ± SD)
Most measured values are under the respective LOQ for each compound.

Table 13: Carbadox, Desoxycarbadox and QCA in Gastric and Intestinal Fluids with liver: Recovery vs time

	Gastric Fluid: % Recovery			Intestinal Fluid: % Recovery		
Time	**Carbadox**	**Desoxy-C**	**QCA**	**Carbadox**	**Desoxy-C**	**QCA**
0 hours	39.3 ± 11.1	114 ± 22	42.7 ± 3.3	34.6 ± 6.9	104 ± 33	26.8 ± 7.0
4 hours	13.5 ± 6.8	38.4 ± 33.3	34.2 ± 5.5	4.6 ± 6.9	74.7 ± 10.3	31.9 ± 10.7

Data expressed as the mean (n=12 ± SD)
Most measured values are under the respective LOQ for each compound

APPRAISAL

As no new data on the genotoxic or carcinogenic nature of carbadox and its metabolites had been generated since the previous evaluation, the Committee was again unable to establish an ADI.

New studies were, however, which were provided at the present meeting supplied information 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 36th 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 are 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 determined quantitatively in liver only up to 48 h, but desoxycarbadox was present in quantifiable concentrations until the end of the study, 15 days after the last administration of medicated feed.
- The Committee at its 36th meeting concluded that more than 90% of the total residues in tissues were bound and could not be extracted 28 days after withdrawal. It agreed that 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. In the new study, the methods included treatment of samples with digestive enzymes (USP systems that mimic gastric and intestinal fluids, respectively). With these techniques, the amounts of desoxycarbadox that could be released from liver tissues were increased by two- to fourfold. The possibility cannot be excluded that, with these techniques, desoxycarbadox could be released at times beyond the 15 days of the duration of the present study.
- The Committee at its 36th 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 residues resulting from the use of carbadox in pigs were acceptable, provided the concentrations 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 desoxycarbadox in liver and also confirmed the time required to deplete QCA to less than 30 µg/kg, they also showed that desoxycarbadox 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 desoxycarbadox. The tolerance limits for the concentration of desoxycarbadox 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 in compliance 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.

New studies

Two new studies were provided, supplying new 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/ton) in combination with 880 mg/kg oxytetracycline. The pigs were treated with carbadox for 28 days and in combination with oxytetracycline for an additional 14 days. Five animals were killed at each of seven times, and their livers were analyzed 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/t 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 enzymatic 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 depleted in liver from 420 ng/kg at 0 h to 50 ng/kg 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 the 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 analyzed 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 deplete quickly, from 22 000 ng/kg at 0 h to 420 ng/kg 96 h after withdrawal.

In a study on carbadox residues in the USA, 34 crossbred 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.

Methods of analysis

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 routinely used in many regulatory laboratories for over a decade.

A new method based on liquid chromatography-atmospheric pressure chemical ionization 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 0.015 mg/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 dexoxycarbadox 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.

Relationship between QCA and carcinogenic residues

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 the 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 36th 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 is not supported for determining 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. 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. Therefore, 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 carbodox in food that would have no adverse health effects in consumers. The Committee decided to withdraw the MRLs of carbadox recommended by the Committee at its 36th meeting.

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DELTAMETHRIN

First draft prepared by

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ADDENDUM

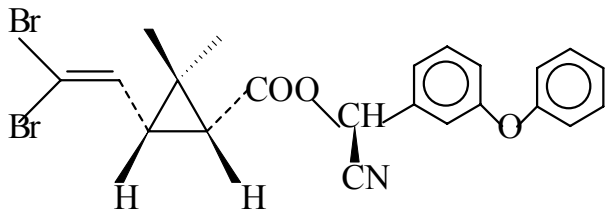
To the monograph prepared by the 52nd meeting of the Committee and published in the FAO Food and Nutrition Paper 41/12

IDENTITY

Chemical Name: S-cyano-3-phenoxybenzyl-*cis*-(1R, 3R) -3-(2,2- dibromovinyl)-2,2-dimethylcyclopropane carboxylate

Synonyms: RU 22974, Decamethrin, Butox®

Structural formula:



Molecular formula: $C_{22}H_{19}Br_2NO_3$

Molecular weight: 505.2

CONDITIONS OF USE

Deltamethrin is an insecticide belonging to the synthetic pyrethroid class and used particularly for control of Diptera and Mallophaga in veterinary use. It is a neurotoxic agent that is widely used for insect control as a pesticide. For veterinary use it is applied topically as a dip, spray or a pour-on preparation to cattle, sheep, pigs, poultry and salmon.

BACKGROUND

Deltamethrin has been evaluated previously by the 52nd Committee for maximum residue limits (MRL) in food animals. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluated deltamethrin toxicologically in 1980, 1981, and 1982 (JMPR 1980, JMPR 1981, JMPR 1982). An Acceptable Daily Intake (ADI) of 0-10 µg/kg of body weight was established at the 1982 meeting. MRLs were recommended for veterinary use in 1990 and for use as a pesticide for plant protection purposes in 1982 by JMPR. MRLs in food animal tissues were recommended by the 52nd meeting of the Committee that affirmed the MRLs for liver, kidney and fat (FAO, 2000). The 52nd meeting of 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 muscle in cattle, sheep, chickens and salmon and for cows' milk and chickens' eggs at 30 µg/kg, expressed as parent drug.

The thirteenth Session of the Codex Committee on Residues of Veterinary Drugs in Food (Codex Alimentarius Commission, 2001) commented with regard to information relevant to the intake of deltamethrin from use as a pesticide and veterinary drug. In particular, some concern was suggested regarding the JECFA MRLs and the possible consideration to amend the MRLs (for veterinary drug residues) to accommodate results of the information on intake, particularly with regard to dietary intake from pesticide use and veterinary use.

NEW INFORMATION

The 2002 meeting of the JMPR evaluated the possible risk associated with the intake of ~~for~~ pesticide residues in food, including those from food animals, using procedures developed in 1999 (JMPR, 2002). Estimates of both, long-term and short term dietary intakes were carried out and expressed as international estimated daily intakes (IEDIs) and international estimate of short-term intake (IESTI). Dietary intakes were calculated by multiplying the concentrations of residue based on supervised trial median residue values (STMRs) with the average daily *per capita* consumption estimated for each commodity on the basis of the WHO Global Environmental Monitoring System (GEMS Food) diet. Long-term dietary intakes were expressed as a percentage of the ADI for a 60 kg person. For dietary intake calculations, JMPR considers that for mammalian animals, 20% of the cattle meat, for example, consumption value-large portion should be considered to contain residues at the concentration amount in fat and that 80% of the meat consumption –large portion would be considered to contain residues at the amount found in meat with trimmable fat removed. For poultry calculations, JMPR use percentages of 10% and 90%, respectively for fat and muscle tissue. This applies to both fat-soluble and non fat-soluble pesticides.. In circumstances when adequate data are not available for theoretical maximum daily intake calculations (for example) the dietary intake calculation would be based on the MRL for meat fat for fat soluble pesticides and the MRL for meat for non-fat soluble pesticides. For deltamethrin, with an ADI of 0-10 µg/kg of body weight, the estimated long-term intake expressed as a percentage of the ADI was ranged between 20-30 percent. The data used for the IEDI (long-term) determination is reprinted below in Table 1 (JMPR, 2002). The following concentration values were used for the estimation of dietary exposure (JMPR, 2002, pp 95ff): fat of cattle: 0.19 mg/kg as the high residue value and 0.16 mg/kg for the supervised trial median residue value; muscle of cattle: 0.027 mg/kg for the high residue level value and 0.01 mg/kg for the STMR. Corresponding residue values for poultry fat were 0.09 mg/kg for the high residue level 0.04 mg/kg for the STMR. The residue values for muscle of poultry were 0.02 mg/kg for both, the high residue level and the STMR. On this basis the percent ADI attributed to the IEDI for meat and poultry is 0-1% depending on the individual GEMS Food diet, while the residues from all sources is no more than 25 percent of the ADI for any of the five regional diets.

For the international estimate of short-term intake (IESTI) in the general population the data are compiled in Table 2. The acute dietary reference dose (RfD) value assigned by JMPR is 0.05 mg/kg body weight. The procedure for calculating the international estimate of short-term dietary intake was first developed by the Geneva Consultation in 1997 and first applied by JMPR in 1999. The calculation for animal commodities was first applied at the 2002 JMPR meeting. For deltamethrin, the IESTI attributed to meat and poultry tissues was 1-2% of the acute RfD. These data are for information only and are not used in estimating dietary exposure ~~of~~ to deltamethrin resulting from the use as a pesticide and a veterinary drug

Table 1. Long-term International Estimated Dietary Intake for Deltamethrin

Commodity	MRL	STMR	Diets: g/person/day. Intake = daily intake: µg/person									
			Mid-East		Far-East		African		Latin American		European	
	mg/kg	mg/kg	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake
Apple		0.03	7.5	0.2	4.7	0.1	0.3	0.0	5.5	0.2	40	1.2
Apple juice		0.0027										
Carrot		0.01	2.8	0.0	2.5	0.0	0	0.0	6.3	0.1	22	0.2
Cattle kidney (2)	0.05		0.1	0.0	0	0.0	0.1	0.0	0.2	0.0	0.2	0.0
Cattle liver (2)	0.05		0.2	0.0	0	0.0	0.1	0.0	0.3	0.0	0.4	0.0
Cereal grain (1)		0.7	56.6	39.6	49.2	34.4	160	112.0	54	37.8	38.6	27.0
Citrus fruit		0.01	54.3	0.5	6.3	0.1	5.1	0.1	54.8	0.5	49	0.5
Eggs (2)		0.02	14.6	0.3	13.1	0.3	3.7	0.1	11.9	0.2	37.6	0.8
Flat bread		0.35										
Flowerhead brassicas		0.02	1.8	0.0	2.5	0.1	0	0.0	1.4	0.0	15.7	0.3
Fruiting vegetables. cucurbits		0.02	80.5	1.6	18.2	0.4	0	0.0	30.5	0.6	38.5	0.8
Grapes		0.04	15.8	0.6	1	0.0	0	0.0	1.3	0.1	13.8	0.6
Hazelnut		0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.3	0.0
Leafy vegetables		0.125	7.8	1.0	9.7	1.2	0.0	0.0	16.5	2.1	51.3	6.4
Leek		0.07	0.5	0.0	0.0	0.0	0.0	0.0	0.3	0.0	2.0	0.1
Legume vegetables		0.01	9.5	0.1	1.5	0.0	0.0	0.0	4.3	0.0	26.0	0.3
Maize germ		0.224										
Maize oil		12.6	1.8	22.7	0	0.0	0.3	3.8	0.5	6.3	1.3	16.4
Meat (from mammals other than marine animals) (2)			37		32.8		23.8		47		155.5	
Meat×0.2 (fat)	0.5	0.16	7.4	3.7	6.56	3.3	4.76	2.4	9.4	4.7	31.1	15.6
Meat×0.8 (muscle)	0.03		29.6	0.9	26.24	0.8	19.04	0.6	37.6	1.1	124.4	3.7
Milks (2)	0.05		116.8	5.8	32	1.6	41.8	2.1	160	8.0	294	14.7
Mushrooms		0.02	0.3	0.0	0.5	0.0	0	0.0	0	0.0	4	0.1
Nectarine		0.02	1.25	0.0	0.25	0.0	0	0.0	0.4	0.0	6.25	0.1
Olive oil. crude		0.315	1.5	0.5	0	0.0	0	0.0	0	0.0	7.8	2.5
Olive oil. refined		0.336	1.5	0.5	0	0.0	0	0.0	0	0.0	7.8	2.6
Olives (used preserved)		0.21	1.3	0.3	0	0.0	0	0.0	0.3	0.1	2.8	0.6

Commodity	MRL	STMR	Diets: g/person/day. Intake = daily intake: µg/person									
			Mid-East		Far-East		African		Latin American		European	
	mg/kg	mg/kg	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake
Onion. bulb		0.02	23	0.5	11.5	0.2	7.3	0.1	13.8	0.3	27.8	0.6
Peach		0.02	1.25	0.0	0.25	0.0	0	0.0	0.4	0.0	6.25	0.1
Plum (includes prune)		0.05	1.8	0.1	0.5	0.0	0	0.0	0	0.0	4.3	0.2
Potato		0.01	59	0.6	19.2	0.2	20.6	0.2	40.8	0.4	240.8	2.4
Poultry meat (2)			31		13.2		5.5		25.3		53	
Poultry fat (meat×0.1)	0.5	0.04	3.1	1.6	1.32	0.7	0.55	0.3	2.53	1.3	5.3	2.7
Poultry muscle (meat×0.9)	0.03		27.9	0.8	11.88	0.4	4.95	0.1	22.77	0.7	47.7	1.4
Poultry. edible offal (2)	0.05		0.1	0.0	0.1	0.0	0.1	0.0	0.4	0.0	0.4	0.0
Pulses		0.5	24.6	12.3	19.8	9.9	17.8	8.9	23.1	11.6	12.1	6.1
Radish		0.01	0.5	0.0	0	0.0	0	0.0	0.3	0.0	2	0.0
Rice. bran (unprocessed)		1.05										
Rice. husked (brown)		0.105	0	0.0	1.8	0.2	34.7	3.6	21	2.2	2.5	0.3
Rice. polished		0.042	48.8	2.0	277.5	11.7	68.8	2.9	65.5	2.8	9.3	0.4
Salmon (2)	0.03		1.3	0.0	5.3	0.2	4.7	0.1	1.3	0.0	1.5	0.0
Sorghum flour		0.231	2	0.5	9.7	2.2	26.6	6.1	0	0.0	0	0.0
Sorghum starch		0.028										
Steamed bread (Dumplings etc)		0.098										
Strawberry		0.02	0	0.0	0	0.0	0	0.0	0	0.0	5.3	0.1
Sunflower seed		0.05	1	0.1	0	0.0	0.6	0.0	0	0.0	0	0.0
Sweet corn (corn-on-the-cob)		0.02	0	0.0	0	0.0	4.4	0.1	0	0.0	8.3	0.2
Tea. green. black (3)		0.0044	2.3	0.0	1.2	0.0	0.5	0.0	0.5	0.0	2.3	0.0
Tomato		0.02	44.4	0.9	5.72	0.1	14.58	0.3	25.5	0.5	40.4	0.8
Tomato paste		0.002	5.8	0.0	0.2	0.0	0.3	0.0	0	0.0	4	0.0
Tomato puree		0.002										
Walnuts		0.02	0	0.0	0	0.0	0	0.0	0	0.0	0.5	0.0
Wheat bran. unprocessed		2.31										
Wheat flour		0.217										
Wheat germ		0.84	0.1	0.1	0.1	0.1	0	0.0	0.1	0.1	0.1	0.1
Wheat wholemeal		0.637	0.3	0.2	0	0.0	0	0.0	0	0.0	0	0.0

Commodity	MRL	STMR	Diets: g/person/day. Intake = daily intake: µg/person									
			Mid-East		Far-East		African		Latin American		European	
	mg/kg	mg/kg	diet	intake	diet	intake	diet	intake	diet	intake	diet	intake
White bread		0.098	215.3	21.1	76	7.4	18.9	1.9	37.3	3.7	117.2	11.5
White noodles		0.091										
Wholemeal bread		0.294	107.7	31.7	38	11.2	9.4	2.8	74.7	22.0	58.6	17.2
Yellow alkaline noodles		0.119										
Total=				151		87		148		107		138
%ADI				25%		16%		25%		18%		23%
Rounded				30%		20%		30%		20%		20%

1. Where residue information were available the consumption of the processed commodities were subtracted for the cereal grain.

2. The 52nd JECFA recommended MRLs for cattle, sheep and chickens in fat at 0.5 mg/kg, liver and kidney at 0.05 mg/kg and 0.05 mg/kg for muscle, eggs and milk at 0.03 mg/kg. An MRL was also recommended for salmon at 0.03 mg/kg. As the JECFA recommendations were the same or higher than the JMPR recommended values and they comprised the major commodities consumed in the commodity group meat mammalian, kidney and liver of cattle, goats, pigs and sheep and for poultry for which JMPR recommendations were made, JMPR decided to utilize the recommendations of the 52nd JECFA for the purposes of estimating dietary intake.

3. The tea STMR was multiplied by the highest processing factor for tea water (brewed tea) = 2.2×0.002

Table 2. Deltamethrin International estimate of short-term intake (IESTI) in the general population

Commodity	STMR or STMR-P, mg/kg	HR, mg/kg	Large portion diet			Unit weight g			Var factor	Case	IESTI, ug/kg bw/day	% acute RfD
			Country	Body weight, kg	Large portion, g	Unit weight g	Country	Edible portion, g				
Apple		0.08	USA	65	1348	138	USA	127	7	2a	2.6	5
Apple juice	0.0027											
Barley	0.7		NLD	63	378					3	4.2	8
Carrot		0.02	NLD	63	336	100	FRA	89	7	2a	0.3	1
Cauliflower		0.04	UK	70.1	579	1733	UK	780	5	2b	1.7	3
Cereal grain (1)												
Chinese cabbage		1.00	USA	65	377	840	USA	798	5	2b	29.0	58
Citrus fruit (2)												
Common beans (pods and or immature seeds)		0.14	NLD	63	431					1	1.0	2
Cucumber		0.02	NLD	65	313	301	USA	286	5	2a	0.4	1
Eggs		0.03	FRA	62,3	219					1	0.1	0

Commodity	STMR or STMR-P, mg/kg	HR, mg/kg	Large portion diet			Unit weight g			Var factor	Case	IESTI, ug/kg bw/day	% acute RfD
			Country	Body weight, kg	Large portion, g	Unit weight g	Country	Edible portion, g				
Flat bread	0.35											
Flowerhead brassicas (3)												
Fruiting vegetables, cucurbits (4)												
Grapes (includes wine)		0.09	AUS	67	1004	125	FRA	118	7	2a	2.3	5
Hazelnut		0.02	AUS	67	70					1	0	0
Kidney of cattle, goats, pigs and sheep (5)		0.05	USA	65	788					1	0.6	1
Leafy vegetables (6)												
Leek		0.1	FRA	62.3	374	100	FRA	50	7	2a	1.4	3
Legume vegetables (7)												
Liver of cattle, goats, pigs and sheep (5)		0.05	USA	65	380					1	0.3	1
Maize germ	0.224											
Maize oil	12.6		NLD	63	43					3	8.6	17
Meat (mammalian) (5)			AUS	67	521							2
meat×0.2 (fat)		0.50	AUS	67	104					1	0.8	
meat×0.8 (muscle)		0.03	AUS	67	417					1	0.2	
Milks (5)	0.03		NLD	63	2515					3	1.2	2
Mushrooms		0.03	FRA	62.3	219					1	0.1	0
Nectarine		0.05	USA	65	590	136	USA	125	7	2a	1.0	2
Olive oil, crude	0.32		FRA	62.3	57					3	0.3	1
Olive oil, refined	0.34											
Olives (used preserved)		0.31	NLD	63	63					1	0.3	1
Onion, bulb		0.03	FRA	62,3	306	140	FRA	126	7	2a	0.5	1
Oranges, sweet		0.01	USA	65	564	131	USA	96	7	2a	0.2	0
Peach		0.05	JAP	52.6	626	122	UK	110	7	2a	1.2	2
Peas, dry												
Plum (includes prune)		0.05	USA	65	413	66	USA	62	7	2a	0.6	1
Potato		0.01	NLD	63	687	122	UK	99	7	2a	0.2	0
Poultry meat (5)			AUS	67	431							1
Poultry fat (meat×0.1)		0.50	AUS	67	43					1	0.3	

Commodity	STMR or STMR-P, mg/kg	HR, mg/kg	Large portion diet			Unit weight g			Var factor	Case	IESTI, ug/kg bw/day	% acute RfD
			Country	Body weight, kg	Large portion, g	Unit weight g	Country	Edible portion, g				
poultry muscle (meat×0.9)		0.03	AUS	67	388					1	0.2	
Poultry, edible offal of (5)		0.03	USA	65	248					1	0.1	0
Pulses (8)	0.5		FRA	62.3	445					3	3.6	7
Pumpkin		0.02	JAP	52.6	218	116	USA	81	7	2a	0.3	1
Radish		0.01	FRA	62.3	204					1	0	0
Rice, bran (unprocessed)	1.05		AUS	67	50					3	0.8	2
Rice, husked (brown)	0.105		JAP	52.6	319					3	0.6	1
Rice, polished	0.042		JAP	52.6	402					3	0.3	1
Salmon, Pacific (5)		0.03	AUS	67	355					1	0.2	0
Sorghum flour	0.231											
Sorghum starch	0.028											
Spinach		1.00	NLD	63	820	111	UK	81	10	2a	24.6	49
Steamed bread (Dumplings etc)	0.098											
Strawberry	0.02	0.10	FRA	62.3	346					1	0.6	1
Sunflower seed		0.05	USA	65	193					1	0.1	0
Sweet corn (corn-on-the-cob)		0.02	USA	65	367	371	UK	215	5	2a	0.4	1
Tea, green, black (9)	0.002		JAP	52.6	16					3	0	0
Tomato		0.20	USA	65	391	123	USA	123	7	2a	3.5	7
Tomato paste	0.002											
Tomato puree	0.002											
Walnuts	0.02		FRA	62.3	136					3	0	0
Watermelon		0.02	USA	65	1939	4518	USA	2078	5	2b	3.0	6
Wheat bran, unprocessed	2.31		USA	65	80					3	2.8	6
Wheat flour	0.217		USA	65	365					3	1.2	2
Wheat germ	0.84		FRA	62.3	207					3	2.8	6
Wheat wholemeal	0.637		USA	65	155					3	1.5	3
White bread	0.098		JAP	52.6	271					3	0.5	1
White noodles	0.091											
Wholemeal bread	0.294		JAP	52.6	277					3	1.5	3
Yellow alkaline noodles	0.119											
											MAX	58

Commodity	STMR or STMR-P, mg/kg	HR, mg/kg	Large portion diet			Unit weight g			Var factor	Case	IESTI, ug/kg bw/day	% acute RfD
			Country	Body weight, kg	Large portion, g	Unit weight g	Country	Edible portion, g				
											IESTI =	

1. The cereal grain commodity with the highest consumption listed was for barley.
2. Calculations were conducted for both the commodity with the highest consumption (oranges) and the commodity with the highest unit weight (grapefruit).
3. See cauliflower
4. Calculations were conducted for both the commodity with the highest consumption (cucumber) and the commodity with the highest unit weight (pumpkin and watermelon).
5. The 52nd JECFA recommended MRLs for cattle, sheep and chickens in fat at 0.5 mg/kg, liver and kidney at 0.05 mg/kg and muscle, eggs and milk at 0.03 mg/kg. An MRL was also recommended for salmon at 0.03 mg/kg. As the JECFA recommendations were the same or higher than those of JMPR, and they comprised the major commodities consumed in the commodity groups, meat mammalian, kidney and liver of cattle, goats, pigs and sheep and for poultry for which recommendations were made, the JMPR decided to utilize recommendations of the 52nd JECFA for the purposes of estimating dietary intake.
6. Calculations were conducted for both the commodity with the highest consumption (spinach) and the commodity with the highest unit weight (Chinese cabbage).
7. The legume vegetable commodity with the highest consumption listed was Common beans.
8. The pulse commodity with the highest consumption was dry peas.
9. The tea STMR was multiplied by the highest processing factor for tea water (brewed tea) = 2.2×0.002.

APPRAISAL

The ADI for deltamethrin is 0-10 µg per kg of body weight, equivalent to 600 µg for a 60 kg person. The data in Table 1 indicate that long term exposure of deltamethrin, using the current approach for estimating pesticide exposure by JMPR (i.e., using supervised trial median residue values) and using the five regional GEMS Food data base food consumption values, residue exposure does not exceed 25 percent of the ADI (ca. 150 µg). While not a component of the estimated dietary exposure of deltamethrin, the exposure of deltamethrin in acute, short term exposure (Table 2) accounts cumulatively to about seven percent of the ADI (ca. 42 µg) from all animal sources of deltamethrin residues (including milk which accounted for about 2%).

The 52nd Meeting of this Committee took account of the previous evaluations of deltamethrin by JMPR and, based on 1) the Committee theoretical daily intake values of 300g of muscle, 100g of liver, 50g of kidney and fat, 1.5 kg of milk and 100g of eggs, and 2) that the marker residue accounted for 4% of the total residues in liver, 3% of the total residues in kidney and 60 percent of the total residues in fat, the most conservative estimate of theoretical maximum daily intake of residues from veterinary drug use would be 250 µg as deltamethrin equivalents. The 250 µg value does not include the recommended guidance MRLs from the 52nd meeting of the Committee for muscle, milk and eggs. Using the guidance MRLs for muscle tissue and milk would add 108 ug of theoretical residues of deltamethrin. There is no information on eggs because residues are well below the limit of quantitation of the method.

The theoretical maximum intake from veterinary use from all food producing animals and animal products has an upper limit of 250 µg (inlcuding the guidance MRL this value would be 358 µg). The sum of theoretical deltamethrin residues from veterinary use and from secondary exposure is no more than 400 µg. This is equivalent to approximately 67 percent of the ADI (under the more conservative scenario which includes the guidance MRL it would be 85 percent of the ADI).

On this basis the committee affirmed the recommended MRLs from the 52nd meeting of the Committee for deltamethrin MRLs in food producing animals and that CCRVDF should be advised accordingly.

REFERENCES

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DICYCLANIL

First draft prepared by

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Dieter Arnold, Berlin, Germany

ADDENDUM

To the dicyclanil residue monograph prepared by the 54th meeting of the Committee and published in FAO Food and Nutrition Paper 41/13 in Rome, 2000

IDENTITY

Chemical name: International Non-Proprietary Name (INN): DICYCLANIL

International Union of Pure and Applied Chemistry (IUPAC) name:

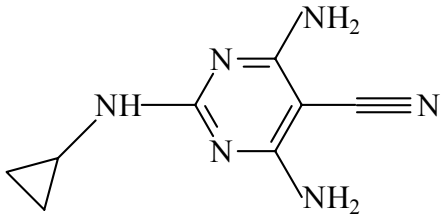
4,6-diamino-2-cyclopropylaminopyrimidine-5-carbonitrile

Chemical Abstract Service (CAS) name:

4,6- diamino-2-(cyclopropylamino)-5-pyrimidinecarbonitrile

Synonyms: A-9568 B, CGA 183893

Structural formula:



CAS number: 112636-83-6

Molecular formula: $C_8H_{10}N_6$

Molecular weight: 190.2

Dicyclanil was first reviewed by the Joint Expert Committee on Food Additives (JECFA) at the 54th meeting of the Committee, which considered its use as an insect growth regulator in sheep. MRLs for muscle, liver, kidney and fat tissues were set.

These MRLs were discussed by the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) at its 13th Meeting. The Committee noted that the use of dicyclanil itself as a marker could result in an estimated total maximum daily intake above the ADI and therefore, recommended that JECFA consider this issue (ALINORM 03/31).

The present re-evaluation of dicyclanil residues was undertaken to address the questions of MRLs relative to ADI, MRL allocation to various tissues, the appropriate marker residue and suitable analytical procedures.

The Committee reviewed all studies that the sponsor had performed in order to investigate the influence of a variety of factors such as breed, type and length of the wool, time of off-shears and the applied dose on the concentrations of dicyclanil related residues in edible tissues. Although Merino sheep were used in most studies, statistical analysis of the results indicated that there were differences among the various breeds in terms of residue kinetics. The other variables among and within the studies were also analyzed and they were considered when indicated. For the purpose of statistical evaluations data obtained for different tissues (e.g. for different types of fat tissues as well as for the muscle tissue from various parts of the body) were pooled when appropriate. The Committee also noted the inconsistency in the metabolic profile established using radiolabel dicyclanil and the observed residues in the subsequent residue depletion studies using non-radiolabel dicyclanil.

RESIDUES IN FOOD AND THEIR EVALUATION

Metabolism

Rats

The previous evaluation by JECFA addressed the studies using ^{14}C-labeled dicyclanil (5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine) in rats by Hassler (1994) and Thanei (1996). These studies showed that orally administered dicyclanil equivalent radioactivity was practically completely recovered in urine and feces. The remaining tissue and carcass residues represented approximately 1% or less of the total administered radioactivity. The following compounds were identified using ^{1}H-NMR, IR and mass spectrometry:

N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide (MET 1U),
5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine (MET 2U = CGA 183893 = dicyclanil),
2-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-3-hydroxy-propionic acid (MET 3U),
2-4,6-triamino-pyrimidine-5-carbonitrile (MET 4U = CGA 297107),
3-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-propionic acid (MET 5U)
Only MET 2U and MET 4U could be unequivocally characterized by mass spectrometry.

Biotransformation was initiated by oxidative cyclopropyl-ring opening at various positions followed by further oxidation. Biotransformation was limited to the cyclopropyl-ring while the cyano-group was metabolically stable. The most significant route was the conversion of the dicyclanil to MET 1U corresponding to 50% of the administered dose. CGA 297107 represented 11% of the excreted metabolites.

Sheep

Altogether 6 studies concerning administration of ^{14}C-labeled dicyclanil to sheep were conducted. The studies of Gifford and Dunsire (1994), McLean and Dunsire (1996) and Anderson and Speirs (1998) concerned the absorption, distribution and excretion of ^{14}C-labeled dicyclanil (Here after, Study 1R, Study 2R and Study 3R, respectively). The studies of Thanei (1996a), Phillips (1996) and Loeffler (1998) were aimed at determination of the nature of the residues (hereafter, Study 4R, Study 5R and Study 6R, respectively). The major findings of the radiolabel-studies are presented in Tables 1 and 2.

Table 1. Mean concentrations of ^{14}C- dicyclanil related radioactivity in tissues and excretion

	Study 1R Gifford & Dunsire	**Study 2R MacLean & Dunsire**	**Study 3R Anderson & Speirs**	
Dose run-off	1.25 g by jetting 37-59%	1.5 g by pour-on 2-10%	100 mg/kg pour-on 22%	
No. of animals	4	4	4	4
Days post-adm	1	7	7	21
Mean Concentrations (±SD)* in				
Liver	0.289 ±0.092	0.513 ±0.101	2.646 ±0.755	1.475 ±0.255
Kidney	0.071 ±0.020	0.077 ±0.013	0.762 ±0.480	0.230 ±0.085
Muscle tenderl.	0.027 ±0.008	0.069 ±0.083	1.013 ±0.370	0.880 ±0.790
Muscle fore	0.034 ±0.020	0.128 ±0.083	0.896 ±0.438	0.503 ±0.202
Muscle hind	0.057 ±0.018	0.165 ±0.145	2.955 ±1.445	0.506 ±0.175
Fat omental	0.052 ±0.031	0.020 ±0.005	0.431 ±0.262	0.208 ±0.077
Fat perirenal	0.038 ±0.005	0.028 ±0.005	0.633 ±0.403	0.068 ±0.050
Fat dorsal sc.	0.262 ±0.199	0.395 ±0.237	19.908 ±19.014	13.844 ±12.181
Fat ventral sc.	0.206 ±0.098	0.482 ±0.336	1.164 ±0.991	1.123 ±0.826
Recovered by 168 hours				
In urine	0.83%	1.58%	1.66%	
In feces	1.05%	2.26%	1.47%	

*mg/kg CGA 183893 equivalents

Only the study 3R used the highest recommended dose (the applied tested dose was twice the commercial dose recommended for the body weight of the animals in the study) and the method that is recommended for application in the field. This was also consistent with the higher radioactivity counts found in the various tissues compared to study 1R and 2R. According to the results about 2-4% of the dose was absorbed during the first 168 hours and 7% by 21 days post-administration. No clear correlation between the administered dose and the counts of radioactivity was seen among the three studies. Dicyclanil related radioactivity was excreted almost equally via urine and feces. The concentration measured in the dorsal subcutaneous fat of the

study 3R was exceptionally high compared with to other tissues and to this tissue in other studies. The concentration of dicyclanil related radioactivity was highest in the liver in study 1R and 2R, but in study 3R the concentrations in the dorsal subcutaneous fat and in hindquarter muscle were higher than the concentration measured in the liver. In study 3R only the concentration in omental fat was below 0.5 mg/kg dicyclanil equivalents 7 days post-administration and in omental fat, perirenal fat and kidney concentration 21 days post-administration. In study 1R, except for the lowest dose, also the "run-off" was highest.

The characterization of the ^{14}C-labeled dicyclanil related radioactivity in tissues is presented in Table 2. Some of the described extraction procedures included a Soxhlet extraction step, which was omitted in the following extractability calculations because the analytical method used in the residue depletion studies with non-radioactive dicyclanil did not use that procedure.

Table 2. Nature of radioactive residues after topical administration of 14C-labeled dicyclanil

	Study 4R Thanei (1996a)	**Study 5R Phillips (1996)**	**Study 6R Loeffler (1998)**	
Source of sample	Gifford & Dunsire (R1)	MacLean & Dunsire (R2)	Anderson & Speirs (R3)	
Number of animals	4	4	4	4
Days post-administration	3	21	7	21
LIVER				
Microwave extraction	YES	NO	NO	NO
Solvents	Acetonitrile/ hexane	Methanol/ hexane	Acetonitrile/ H_2O/SPE	Methanol/ H_2O/SPE
Extractability	50.3%	20%	64.2%	40.5%
MET 1U	N.D.	N.D.	2.9%	7.9%
MET 2U	(2.7%*)	N.D	18.7%	11.5%
MET 3U	N.D.	N.D.	N.D.	N.D
MET 4U	13.9%	N.D.	15.7%	19.0%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	33.7%	20%	26.9%	41.7%
KIDNEY				
Microwave extraction	YES	NO	NO	NO
Solvents	Methanol/ hexane	Methanol/ hexane	Acetonitrile/ H_2O	Methanol/H_2 O
Extractability	77.2%	58%	91.5%	68.6%
MET 1U	N.D.	N.D.	3.1%	13.4%
MET 2U	(20.1%*)	N.D.	24.4%	22.0%
MET 3U	N.D.	N.D.	N.D.	N.D.
MET 4U	17.4%	N.D.	21.2%	9.5%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	62.5%	42%	51.3%	55.1%
MUSCLE				
Microwave extraction	YES	NO	NO	NO
Extraction solvents	Acetonitrile/ methanol/hexane	Methanol/ hexane	Acetonitrile/ methanol/ H_2O	Acetonitrile/ methanol/ H_2O
Extractability	90.6%	94%	99.7%	98.9%
MET 1U	5.8%	N.D.	3.5%	0.7%
MET 2U	61.9%	67.8%	83.7%	86.0%
MET 3U	N.D.	N.D.	N.D.	N.D.
MET 4U	6.3%	N.D.	4.3%	2.9%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	23.3%	26.3%	8.5%	10.4%
FAT				
Microwave extraction	YES	YES	NO	NO
Solvents	Acetonitrile/	Acetonitrile/hex	Methanol	

	Study 4R Thanei (1996a)	Study 5R Phillips (1996)	Study 6R Loeffler (1998)	
	hexane	ane		
Extractability	95.9%	89%	96.9%	98.0%
MET 1U	N.D.	N.D.	0.8%	0.6%
MET 2U	90.2%	83.6%	90.7%	86.0%
MET 3U	N.D.	N.D.	N.D.	N.D.
MET 4U	2.7%	N.D.	1.3%	0.8%
MET 5U	N.D.	N.D.	N.D.	N.D.
Unresolved and/or unidentified	3.0%	5.5%	5.9%	12.6%
URINE				
Microwave extraction	NO	NO	N.I.	N.I.
Extraction solvents	None	None	N.I.	N.I.
Extractability	100%			
MET 1U	21.0%	N.D.	N.I.	N.I.
MET 2U	32.2%	69.4%	N.I.	N.I.
MET 3U	N.D.	N.D.	N.I.	N.I.
MET 4U	13.2%	9.3%	N.I.	N.I.
MET 5U	5.7%	N.D.	N.I.	N.I.
Unresolved and/or unidentified	24.6%	21.3%	N.I.	N.I.
FECES				
Microwave extraction	NO	NO	N.I.	N.I.
Extraction solvents		Methanol, Methanol:water	N.I.	N.I.
Extractability	90.7%	91%		
MET 1U	2.9%	N.D.	N.I.	N.I.
MET 2U	75.2%	85.4%	N.I.	N.I.
MET 3U	N.D.	N.D.	N.I.	N.I.
MET 4U	7.7%	N.D.	N.I.	N.I.
MET 5U	N.D.	N.D.	N.I.	N.I.
Unresolved and/or unidentified	4.9%	5.6%	N.I.	

N.D. = Not detected
N.I. = Not investigated
SPE = Solid Phase Extraction
* = no definitive separation from MET 1U

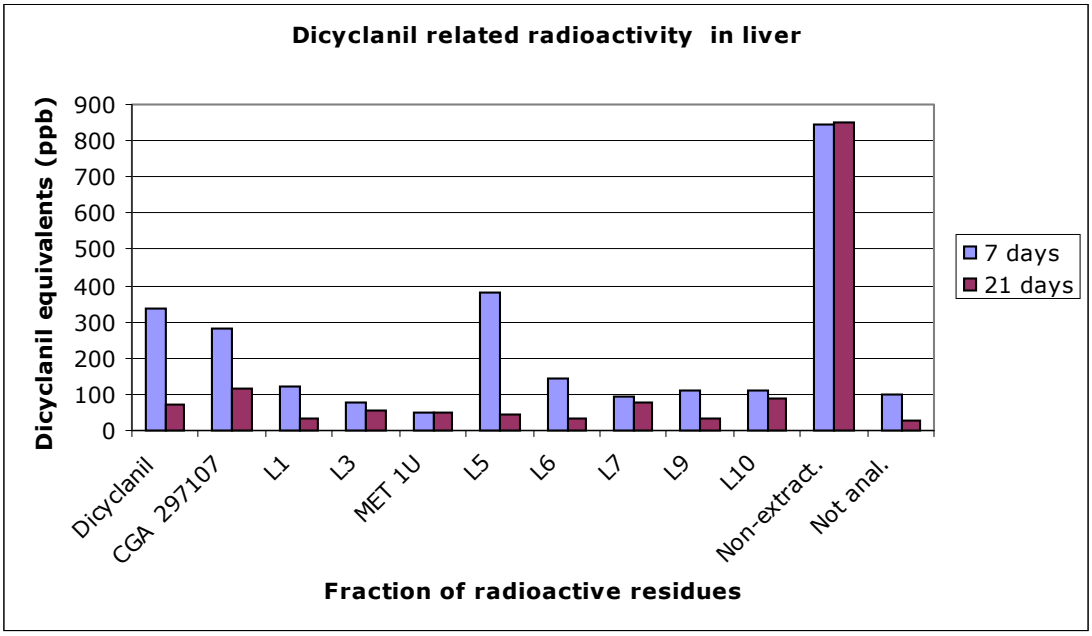
The study 4R used microwave assisted extraction procedure, which is not part of either of the two analytical methods described here. It appears, therefore, unlikely that the results of this study would accurately characterize the extractability or the ratio of the identified metabolites found in the residue depletion studies. Moreover, according to Thanei (1996a), there appears to be a clear trend of other (more unstable) metabolites to break down to CGA 297107 under harsh extraction conditions (microwave and Soxhlet treatment). As indicated above, the excreted amount represented the amount absorbed (2-4% of the dose retained on the animal). Therefore, the amount absorbed in study 1R would not exceed 16 mg while in study 2R the amount absorbed would be up to 57mg.

Of the excreted amount, 56-80% represented the parent compound, dicyclanil, while only 10.2% in study 1R/4R and 4.9% in study 2R/5R consisted of the CGA 297107 metabolite. Therefore, dicyclanil was not extensively metabolized in sheep and CGA 297107 appeared to be a minor metabolic product in excreta. Results of the nature of the residues in urine and feces of study 3R/6R were not available. However, the two earlier studies appeared sufficient to clarify this aspect. Study 3R/6R is the radiolabel study that appeared to best address the characterization of dicyclanil related metabolism in sheep. Furthermore, the application mode was identical and the analytical extraction procedure comparable to the described analytical methods used in the non-radiolabel studies.

In study 3R/6R, dicyclanil represented 18.7% and CGA 297107 15.7% of the extractable residues in the liver 7 days post-administration. The respective concentrations 21 days post-administration were 11.5% and 19%. Another 2.9% of the extractable residues were characterized as MET 1U 7 days and 7.9% 21 days post-administration. Over 60% of the extractable radioactive residues in liver could not be identified. In the study 1R/4R dicyclanil was not unequivocally identified in the liver (possibly 4.4%) and the only metabolite that could be characterized in that study was the CGA 297107. In the study 2R/5R

only dicyclanil was identified while CGA 297107 was not detected. Figure 1 shows the concentration of the various components as a function of time in study 3R/6R in liver tissue. The non-extractable residue concentration appeared to remain practically constant over time. The same trend was seen also in studies 1R/4R and 2R/5R. Dicyclanil concentration declined somewhat faster than CGA 297107 concentration. However, some of the unidentified fractions declined slower than CGA 297107. The changing ratio of the extractable and non-extractable residue concentration may bias the calculation of total residues when factors are used.

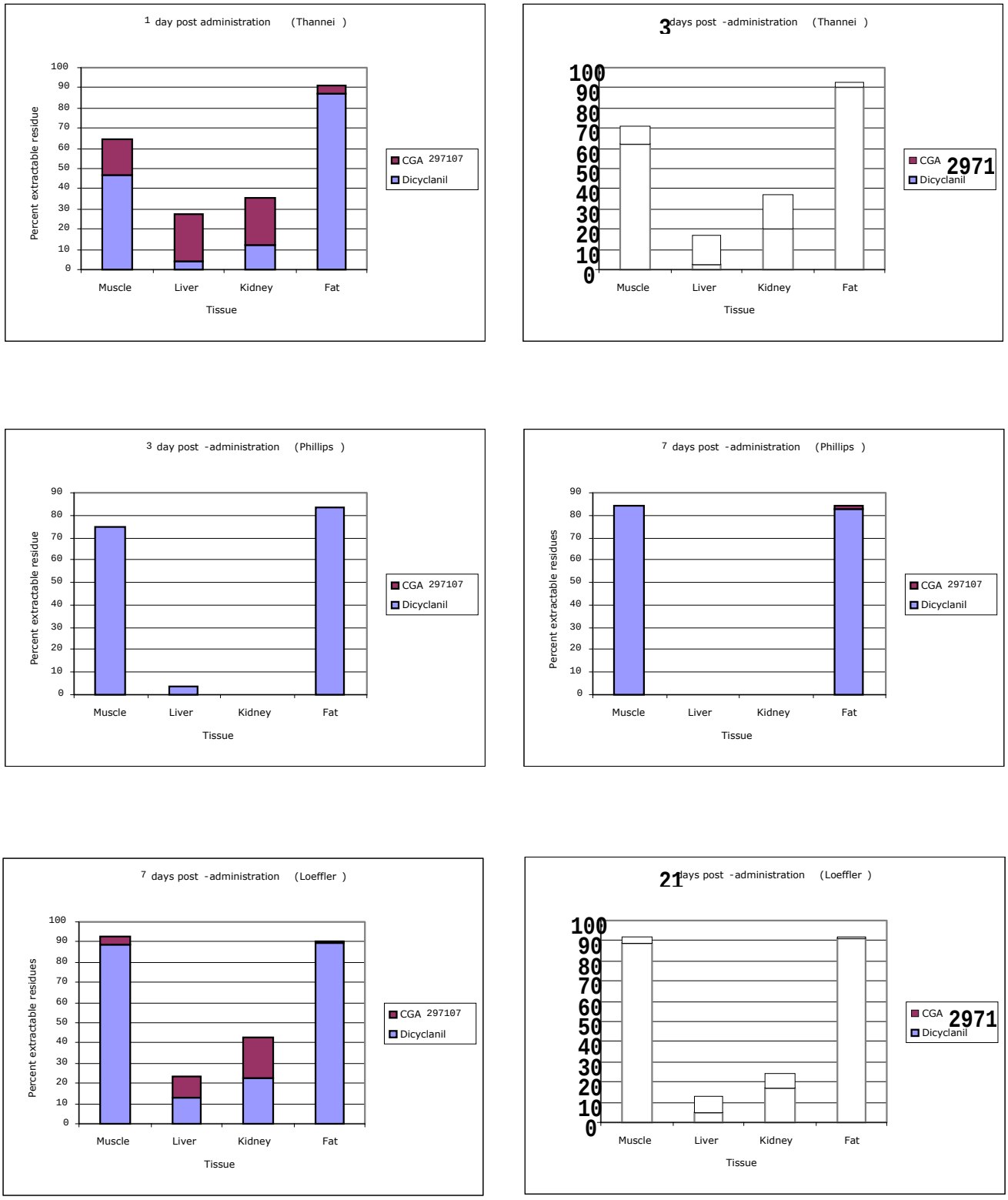
Figure 1. Concentration and identification of dicyclanil related radioactivity in liver tissue as a function of time after pour-on administration of dicyclanil at 100 mg/kg (Loeffler, 1998)



In the kidney, dicyclanil related total radioactivity was low at 7 days post-administration (0.723 mg/kg dicyclanil equivalents). The extractable residues consisted of 24.4% dicyclanil and 21.2% CGA 297107 in HPLC analysis. The respective values at 21 days post-administration were 22.0% and 9.5% but the extractability decreased. As in liver also in kidney the concentration of non-extractable residues did not change as a function of time. At 21 days post-administration total dicyclanil related residues were 0.230 mg/kg dicyclanil equivalents. In the kidney the MET 1U represented 3.4% of the extractable residues. As in the liver, a high proportion of the extractable radioactive residues could not be identified in kidney either. In the Study 1R/4R the kidney metabolite profile was almost identical to that in the liver. If the metabolite unresolved from MET 1U would be dicyclanil, the profile would be similar to that in the subsequent studies.

As indicated in the previous evaluation, the major component in muscle and fat tissues was dicyclanil (Figure 2). The CGA 297107 contributed only nominally (less than 5%) to the total extractable radioactive residues. In the study 1R the extractability was lower than in the two other studies while the CGA 297107 also appeared in higher proportion in that study. However, only studies 2R and especially 3R included time points of sampling that were relevant to the subsequent residue depletion studies. Unfortunately none of the studies provided information beyond 21 days post-administration.

Figure 2. Percentage of extractable dicyclanil and its metabolite CGA 297107 residues in muscle, liver, kidney and fat tissues after topical administration of dicyclanil at 1.25 g (Thanei, 1996a), 1.5 g (Phillips, 1996) and 100 mg/kg (Loeffler, 1998)



As can be seen from Figure 2, in all studies performed using radioactive dicyclanil, the extractable muscle and fat tissue residues were almost exclusively dicyclanil.

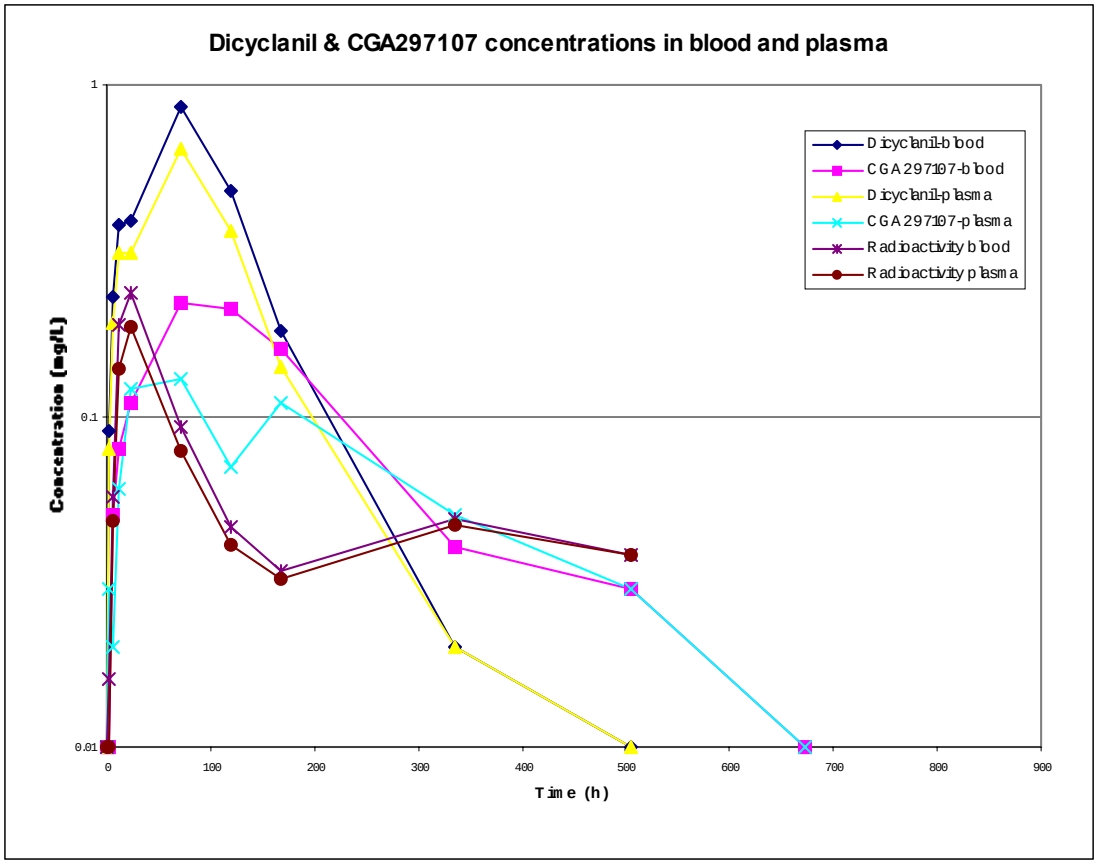
TISSUE RESIDUE DEPLETION STUDIES

Residue Depletion Studies (with Unlabelled Drug)

The Committee reviewed eight studies which were performed in sheep. Three of these studies were GLP non-compliant and the five other studies were done in accordance to the current GLP standards. Different parameters were investigated including formulation, dose, application method, age, breed and wool length. Furthermore, the studies were not identical in terms of compounds (parent and metabolites) analyzed. These studies were described in detail in the previous evaluation. For the present evaluation, the tissue residue depletion data were analyzed statistically using the logarithms of the concentrations in the various tissues and linear regression. For this purpose data obtained for different tissues, e.g., different types of fat tissues as well as the muscle tissues from various parts of the body, were pooled when appropriate. Thereafter, statistical tolerance limits were calculated as the one-sided upper 95% confidence limit over the 95th percentile of the population.

Figure 3 demonstrates the blood and plasma dicyclanil and CGA 297107 concentrations from the study of Hotz (1999) compared to the dicyclanil related radioactivity expressed as dicyclanil equivalents in blood and plasma by Anderson and Speirs (1998).

Figure 3. Concentrations of dicyclanil and CGA 297107 in blood and plasma after topical administration of non-radiolabelled dicyclanil (Hotz, 1999) compared to the respective total dicyclanil related radioactivity after topical administration of radiolabelled dicyclanil (Loeffler, 1998)



The blood and plasma concentrations appeared much higher in the non-radiolabel study. The dicyclanil concentration was also considerably higher than the CGA 297107 concentration. The half-life of dicyclanil seemed shorter than the half-life of the CGA 297107.

All of the residue depletion studies were considered. Extensive statistical analysis showed that the studies of Peterson and George (1997) in Merino sheep and Hotz (1999) in White Alp sheep adequately represented the depletion of dicyclanil related residues in sheep. The highest residue concentrations in muscle, liver and kidney tissues were recorded in Merino sheep and, therefore, considered representative for the depletion pattern. For the same reason the fat tissue of the White Alp sheep depletion data were used. Variations in the ratio of dicyclanil and CGA 297107 were seen in fat tissues collected from various parts of the animals.

For the present evaluation concerning the dicyclanil and CGA 297107 residues the major features of the study of Peterson and George are presented in Table 3. Only one dose is presented here. The full description of this study was provided in the previous evaluation. The results of the study by Hotz are presented in Tables 4 and 5.

Table 3. Mean (±standard deviation) residues of dicyclanil and CGA 297107 in tissues of Merino sheep treated with dicyclanil at 4mL/kg 0 day off-shears (Trial 97/4/1559 by Peterson and George, 1997)

	Mean ±SD concentrations (mg/kg)*				
Post treatment	**7 days**	**14 days**	**21 days**	**26 days**	**56 days**
Fat Renal					
Dicyclanil	0.16 ±0.03	0.06 ±0.02	0.03 N.C.**	0.03 N.C.	<0.01 N.C.
CGA 297107	0.04 ±0.02	0.01 N.C.	0.01 N.C.	0.01 N.C.	<0.01 N.C.
Subcut.					
Dicyclanil	0.24 ±0.09	0.89 ±1.60	0.05 ±0.04	0.04 ±0.04	0.02 N.C.
CGA 297107	0.05 ±0.03	0.03 ±0.01	0.03 ±0.01	0.02 N.C.	<0.01 N.C.
Muscle					
Dicyclanil	0.80 ±0.43	0.34 ±23	0.20 ±0.20	0.14 ±0.17	0.02 N.C.
CGA 297107	0.48 ±0.07	0.11 ±0.02	0.12 ±0.06	0.10 ±0.09	0.03 ±0.01
Kidney					
Dicyclanil	0.94 ±0.54	0.33 ±0.32	0.22 ±0.23	0.18 ±0.23	0.02 N.C.
CGA 297107	0.41 ±0.19	0.24 ±0.15	0.34 ±0.14	0.26 ±0.16	0.06 ±0.02
Liver					
Dicyclanil	1.21 ±0.69	0.46 ±0.39	0.32 ±0.35	0.22 ±0.26	0.02 N.C.
CGA 297107	0.49 ±0.11	0.23 ±0.05	0.37 ±0.14	0.18 ±0.23	0.08 ±0.01

* Each concentration represents the data of 4 animals

**N.C. = not calculated

Table 4. Mean residues of dicyclanil (CGA 183893) and CGA 297107 in tissues of White Alp sheep treated with dicyclanil at 2mL/kg 1 day and 7 weeks off-shears (Trial 99/17 by Hotz, 1999)

	Mean ±SD concentrations (mg/kg)*							
Post treatment	**7 days**		**14 days**		**21 days**		**35 days**	
Time off-shears	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**
Fat								
Oment. Dicyclanil	0.415	0.365	0.185	0.280	0.138	0.128	0.055	0.067
CGA 297107	0.008	0.018	0.012	0.005	0.005	0.005	0.005	0.005
Renal								
Dicyclanil	0.042	0.033	0.019	0.020	0.033	0.008	0.008	0.005
297107	0.019	0.024	0.005	0.008	0.005	0.009	0.005	0.005
Subcut. Dicyclanil	0.363	0.248	0.223	0.298	0.157	0.090	0.045	0.068
Remote 297107	0.022	0.021	0.008	0.008	0.006	0.005	0.005	0.005
Subcut. Dicyclanil	0.040	0.030	0.017	0.008	0.011	0.011	0.008	0.008
Adm. 297107	0.017	0.013	0.006	0.007	0.005	0.008	0.005	0.005
Muscle								
Hind- Dicyclanil	0.080	0.083	0.026	0.012	0.021	0.008	0.005	0.005
CGA 297107	0.065	0.0072	0.032	0.027	0.075	0.017	0.007	0.011
Fore- Dicyclanil	0.087	0.085	0.026	0.013	0.023	0.008	0.006	0.005
CGA 297107	0.068	0.080	0.042	0.028	0.031	0.020	0.008	0.009
Tender- Dicyclanil	0.087	0.087	0.026	0.013	0.020	0.008	0.006	0.005
CGA 297107	0.067	0.081	0.038	0.028	0.028	0.017	0.008	0.011
Kidney								
Dicyclanil	0.077	0.081	0.023	0.012	0.022	0.011	0.005	0.005
CGA 297107	0.165	0.0195	0.067	0.053	0.057	0.062	0.018	0.028
Liver								
Dicyclanil	0.127	0.127	0.038	0.025	0.033	0.019	0.007	0.005
CGA 297107	0.257	0.237	0.103	0.087	0.077	0.062	0.025	0.032

* Each concentration represents the mean of 6 animals

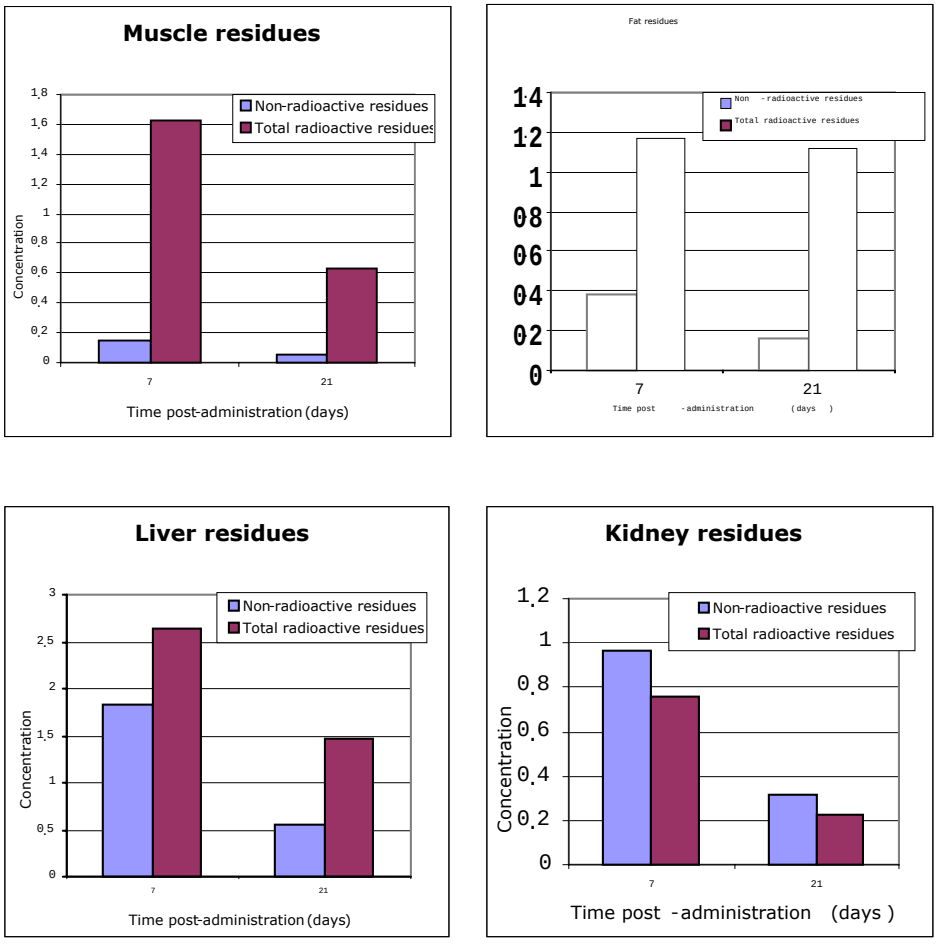
Table 5. The ratio of the mean residues of dicyclanil and the sum of dicyclanil and CGA 297107 ([dicyclanil]/[dicyclanil + CGA 297107]) in tissues of White Alp sheep treated with dicyclanil at 2mL/kg 1 day and 7 weeks off-shears (calculated from Trial 99/17)

	Ratio dicyclanil/(dicyclanil + CGA 297107)							
Post treatment	**7 days**		**14 days**		**21 days**		**35 days**	
Time off-shears	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**	**1 day**	**7 wks**
Fat Oment.	0.98	0.95	0.94	0.98	0.97	0.96	0.92	0.93
Renal	0.69	0.58	0.79	0.71	0.87	0.47	0.62	0.50
Subcut. remote	0.94	0.92	0.97	0.97	0.96	0.95	0.90	0.93
Subcut. Adm.	0.70	0.70	0.74	0.53	0.69	0.58	0.62	0.62
Muscle Hind-	0.55	0.92	0.41	0.31	0.22	0.32	0.42	0.31
Fore-	0.56	0.52	0.38	0.32	0.43	0.29	0.43	0.36
Tender-	0.56	0.52	0.41	0.32	0.42	0.32	0.43	0.31
Kidney	0.32	0.81	0.26	0.18	0.28	0.15	0.22	0.15
Liver	0.33	0.35	0.27	0.22	0.30	0.23	0.22	0.14

The residue concentrations in the study of Peterson and George were generally higher than those reported in the study of Hotz. According to the values given in Table 4, the combined concentration of dicyclanil and CGA 297107 exceeds the MRL suggested by the Committee for dicyclanil only in fat tissue 14 day or more post-administration. The concentrations of both compounds in all tissues appeared to decrease as a function of time. No effect concerning time off-shears could be identified. The ratio of dicyclanil to the sum of dicyclanil and CGA 297107 decreased as a function of time as shown in Table 5.

There appeared to be some inconsistencies between the results obtained in the non-radiolabel and in the radiolabel studies (particularly 3R/6R). All the studies performed with non-radioactive dicyclanil, and in which the CGA 297107 concentration was also determined, appeared to produce similar results. In the study 6R only 0.7-1.5% of the residues in fat was CGA 297107, while the study by Hotz found CGA 297107 up to 53% in perirenal fat and up to 47% in the administration site subcutaneous fat. The ratio in omental and subcutaneous remote site fat, however, appeared to agree with Study 6R. In the muscle tissue 7 days post administration, the concentration of CGA 297107 represented almost 50% of the radioactive residue while in study 6R only 4.3% of the radioactive residue could be attributed to CGA 297107. In the study by Hotz the proportion of CGA 297107 increased with time. In the kidney tissue, except for one value, the concentration of CGA 297107 represented more than 68% of the total residues while the study 6R

Figure 3. Comparison of tissue dicyclanil residues after administration of radiolabel dicyclanil (Loeffler, 1998) and non-radiolabel dicyclanil (Hotz, 1999) to sheep at 100 mg/kg pour-on. The non-radiolabel residues consist of dicyclanil and CGA 297107. Correction factors of 0.15 and 0.25 (suggested by the sponsor) were applied for liver and kidney for the combined dicyclanil and CGA 297107 concentrations



suggested practically equal concentrations of the two compounds. In the liver tissue the study by Hotz found 35% or less of dicyclanil while the study 6R indicated that the concentration of dicyclanil was higher than the CGA 297107. According to the results of Hotz, the tissue of concern would be fat because dicyclanil concentrations exceeding the proposed MRLs can be found only in fat tissue in the observation period exceeding 7 days. The dicyclanil concentrations were high only in the specific fat tissues where the residue appeared to be dicyclanil only. The tissue concentrations in study 3R were higher compared to the results of Hotz, except for kidney tissue, regardless of the tissue when compared to the present study (Figure 3). Based on the available data, it is not possible to explain the discrepancy.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The method was described in the previous evaluation. The document, Summary Report EMEA/MRL/573/99-Rev.1 (1999), of which only parts could be included in the previous evaluation due to time limitations, incorporate important data concerning the analytical method. The submitted data also contained chromatograms obtained by use of the recommended analytical method. The analytical method was validated according to all the requirements of Volume VI of the rules governing medicinal products in the EU for all sheep tissues with both substances.

Dicyclanil and CGA 297107 are analyzed by a method that uses two different HPLC procedures for compound separation and detection. The extraction procedure is identical, using the same sample, until the final elution where dicyclanil is eluted using 1% isopropyl alcohol in dichloromethane while the CGA 297107 is eluted using 25% isopropyl alcohol in dichloromethane. These compounds are eluted from a strong anion exchange solid phase extraction (SPE) cartridge using organic solvents. The compounds here are cations and elution from such cartridges are generally performed with aqueous solutions using changes in the pH as the eluting factor. Therefore, the cartridges are used here as in direct phase separation. The two fractions are injected separately to two separate HPLC systems. The method description contains a warning indicating that the two fractions contain compounds that may interfere with the signal of dicyclanil or vice versa. Although validated for sheep tissues, significant modifications, including extraction, elution and mobile phase comnposition, were made in the most recent residue depletion study by Hotz (1999).

The determinative method applies strong cation exchange column while the described confirmatory method utilizes C18 column for dicyclanil and NH_2 column (with CN guard column) for CGA 297107 analysis. The confirmatory method does not fulfill the criteria of specificity compared to techniques such as mass spectrometry. There was a good agreement between the determinative and confirmatory method concerning dicyclanil while the agreement was poor for CGA 297107 in muscle and fat tissue samples. The limit of quantification for CGA 297107 was set at 100 µg/kg because of a large interfering peak in the confirmatory method. Based on the documentation the confirmatory method for dicyclanil appeared to exhibit best chromatographic performance.

An attempt was made to compare the analytical method and the concentration determined earlier by radioactivity detection (Smal, 1999). It was not clear from the submitted data how the expected ratio and concentrations of dicyclanil and CGA 297107 in the samples were determined. Dicyclanil was not detected in liver and kidney samples that contained 300 and 90 µg/kg dicyclanil related radioactivity. CGA 297107 was not detected in muscle and fat samples containing 80 and 180 µg/kg dicyclanil related radioactivity, respectively.

The use of two components, dicyclanil and its metabolite CGA 297107, as marker residue was suggested by the sponsor. Such an approach is not free of problems. Because the ratio between the components in this case is not constant, a significantly lower limit of quantification (LOQ) of the analytical method is required for both components. The recovery of both components should be independent of the concentration and similar in all tissue types. There should be no interference between the two analytes. Analytical reference material should be readily available for both compounds. The quality assurance procedures must control simultaneously both analytical processes. The cumulative effect of two analytes on precision and accuracy of the total residues must be calculated. The need to use two separate HPLC systems for the analysis of the two compounds was also considered a disadvantage. Based on the radiolabel studies, there seems to be no justification for use of the two components as marker residue for fat and muscle tissue. The proposed HPLC residue control method, however, indicated presence of CGA 297107 in muscle and fat tissues that cannot be explained based on the radiolabel dicyclanil studies. The use of sum of two components as marker residue could be appropriate in the case of liver and kidney. The problem in this case is that the sum of these two components does not form even 50% of the total residue in these tissues. Therefore, it is questionable whether the use of the sum of these two components provides additional accuracy to the determination of the total residues. Because CGA 297107 is a minor metabolite, physiological/pathological fluctuations in its concentration may cause misinterpretation in the total residue concentration. It appears, therefore, that the use of dicyclanil as the only marker residue should be preferred.

APPRAISAL

Dicyclanil was reviewed by the Committee at its 54th Meeting. Data were provided on the use of dicyclanil applied as a pour-on to sheep. Most of the studies were conducted according to current GLP standards. In its previous review, the Committee suggested that dicyclanil should be used as the marker residue. Concern was expressed that the consequence would be that the MRLs would exceed the TMDI. JECFA was requested to clarify its recommendation to use dicyclanil as the marker residue instead of the sum of dicyclanil and its metabolite CGA 297107.

Dicyclanil appeared to be considerably less metabolized in sheep than in laboratory animals. Radiolabel studies indicated that CGA 297107 was a minor dicyclanil metabolite consisting of not more than 5-10% of the excreted dicyclanil related radioactivity, which was consistent with the dicyclanil metabolism reported in laboratory animals.

According to the radiolabel studies dicyclanil is the major residue in muscle and fat tissues. Dicyclanil and CGA 297107 could be found in liver and kidney in almost equal concentrations but not exceeding 50% of the total residues.

The depletion of dicyclanil related residues from tissues was studied extensively and determined as a function of application technique, dose, wool length, sex, breed and age differences. The results of these studies were summarised in the documents produced by the 54th Meeting of the Committee. Most of the studies were conducted using higher than the recommended label doses. Although Merino sheep were used in most studies, statistical analysis in the present evaluation indicated that there were differences among the various breeds in terms of residue kinetics. The other variables among and within the studies were also analyzed and they were considered when indicated. The highest residue concentrations in muscle, liver and kidney tissues were recorded in Merino sheep and, therefore, considered representative for the depletion pattern. For the same reason the fat tissue of the White Alp sheep depletion data were used.

For the present evaluation, the tissue residue depletion data were analyzed statistically using the logarithms of the concentrations in the various tissues and linear regression. For this purpose data obtained for different tissues, e.g., different types of fat tissues as well as the muscle tissues from various parts of the body, were pooled when appropriate. Thereafter, statistical tolerance limits were calculated as the one-sided upper 95% confidence limit over the 95th percentile of the population.

The only available study that could be used for the determination of the ratio between total residue and marker residues was done using Dorset sheep included data until 21 days post-administration. The statistical analysis of the data examined the predictability of total residues when dicyclanil was used alone or together with CGA 297107 as the marker residue. The results showed that for time periods up to 21 days post-administration the variability of the data was only slightly smaller when the sum of the two compounds was used. However, the estimates of theoretical maximum daily intakes using either approach were similar. The Committee considered the use of a single compound approach to be preferred for several reasons. The most important was the need to analyze dicyclanil and CGA 297107 in two separate HPLC runs. This would place an unnecessary burden for a residue control program.

An estimate of a TMDI on the basis of the ratio of marker to total residue was only possible for the time period up to 21 days due to limited data available from the radiolabel study. However, the Committee has attempted to propose MRLs that further limit the exposure of consumers to residues, and therefore, MRLs were reduced to concentrations of dicyclanil that were consistent with good practice in the use of veterinary drugs and which could be determined with practical analytical methods. Estimates of TMDI could not be given for these MRLs since the corresponding concentrations of residues would be reached after approximately 28-32 days following the application of the dose. However, the Committee assumed that at such a late time after the treatment of the animals the parent drug and the metabolite CGA 297107 were the only residue of concern. Sufficient data were available to estimate the concentrations of these two compounds at 28-32 days following treatment with reasonable statistical certainty. Therefore a TMDI could be estimated using the statistical tolerance limits calculated for the sum of dicyclanil and CGA 297107.

Two analytical methods were described. They allowed separate detection of dicyclanil and its CGA 297107 metabolite. Significant amendments had to be made in the determinative method in the most recent residue depletion study. The Committee considered the second method, described as the confirmatory method, for dicyclanil best suited for monitoring in a routine residue control program.Two analytical methods were described. They allowed separate detection of dicyclanil and its CGA 297107 metabolite. Significant amendments had to be made in the determinative method in the most recent residue depletion study. The Committee considered the second method, described as the confirmatory method, for dicyclanil best suited for monitoring in a routine residue control program.

The following points were considered in setting the MRL:

- An ADI of 0 - 0.007 mg/kg of body weight, based on a toxicological endpoint, was recommended which resulted in a maximum daily intake of 0.42 mg for a 60 kg person.
- The marker residue is the parent dicyclanil.
- The total residue of concern at time points beyond 28 days after treatment of the animals is the sum of dicyclanil and its metabolite CGA 297107.
- Dicyclanil residues can be detected using liquid chromatography (HPLC) at the limit of quantification of 0.01 mg/kg.

Estimates of residue intake are tabulated as follows:

Food commodity	MRL (µg/kg)	Concentration of total residue of concern[1)] (µg/kg)	Consumption (g/person/day)	Intake (µg/person/day)
Liver	125	340	100	34
Kidney	125	340	50	17
Muscle	150	230	300	69
Fat	200	200	50	10
Sum				130

[1)] The the upper limit of the 95% confidence interval for the 95th percentile of the sum of the concentrations of Dicyclanil and metabolite CGA297107, expressed as equivalents of dicyclanil

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues in sheep, expressed as the parent drug:

Muscle - 0.15 mg/kg

Liver - 0.125 mg/kg

Kidney - 0.125 mg/kg

Fat - 0.20 mg/kg

Based on consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat, the theoretical maximum daily intake of dicyclanil residues from veterinary use 130 µg/person/. The Committee did not consider dicyclanil use in lactating sheep.

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FLUMEQUINE

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ADDENDUM

To the monographs prepared by the 48th and 54th meetings of the Committee published in the FAO Food and Nutrition Paper 41/10, Rome 1998 and 41/13, Rome 2000, respectively.

IDENTITY

Chemical name:	9-Fluoro-6,7-dihydro-5-methyl-1-oxo-1 H,5H-quinolizine-2-carboxylic acid
Synonyms:	R-802, Apurone
Molecular formula:	$C_{14}H_{12}NFO_3$
Molecular weight:	261.26
Pure active ingredients:	Flumequine
Appearance:	White microcrystalline powder
Melting point:	253-255°
Solubility:	Soluble in aqueous alkaline solutions and alcohol, insoluble in water

INTRODUCTION

Residues of flumequine were evaluated by the Committee at the 42nd (WHO 1998), 48th (WHO 1998) and in the 54th meeting (WHO 2001). The Committee established an ADI of 0-30 µg/kg of body weight based on a toxicological end-point (hepatotoxiciy in male CD-1 mice in the 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 their natural proportions.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Flumequine is a quinolone with antimicrobial activity against Gram negative organism and is used for the treatment of enteric infections in domestic species. It has also a limited use in the treatment of urinary tract infection in man.

Disposition and residues of flumequine in black tiger shrimp

Data was submitted to the Committee for its consideration including: the disposition and residue pattern of flumequine in black tiger shrimp (Penaeus monodon) and for the establishment of MRL in giant prawn or black tiger shrimp. In response to a question from the Committee the sponsor indicated that there is no recommended dose of flumequine for giant prawns.

The disposition and residue data were generated with shrimps (Penaeus monodon) with an average weight of 20-30 gm which were maintained in 5 x 10 m concrete tanks in the open with shading to bring the water temperature to 28-32 grades centigrade at a pH of 8.0. Flumequine was administered to the shrimps at 12 mg/kg shrimp bodyweight by intramuscular injection, forced oral dosing using feeding needle, or mixed to the pelleted feed and given ad libitum for 5 consecutive days.

After drug administration, nine shrimp samples were randomly, collected at 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336 and 360 hours post-dosing. In the groups given feed treatment, shrimps were sampled at daily interval from the tanks, before medicated feed was administred in the morning for a 15 day period.

Samples of the whole edible part of muscle tissue from each shrimp were packed in plastic bag and kept frozen at -20 grades centigrade until assay.

Injection, single oral administration

The absorption and excretion of flumequine in black tiger shrimp following a single intramuscular and oral administration are shown in figure 1. The maximum peak concentration obtained in shrimp muscle, 2616.45 µg/kg at 2 hours following injection and decreased below LOQ at 216 hours post dosing and the maximum peak concentration obtained in shrimp muscle, 365.8 at twelve hours after oral administration and decreased below LOQ after 144 hours post dosing. The mean drug concentrations versus time are presented in table 1.

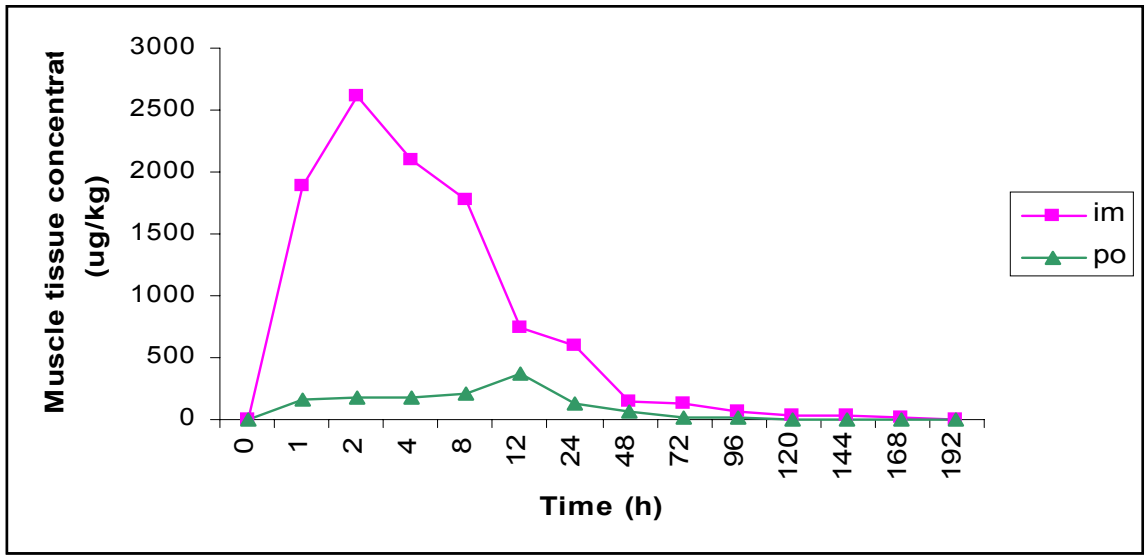
Table 1. Flumequine concentration in shrimp muscle after intramuscular and oral administration of a single dose of 12 mg/kg shrimp.

Flumequine concentration (µg/kg) by HPLC		
Time (hours) post dosing	**Intramuscular [a]Mean (SD)**	**Oral dosing [a]Mean (SD)**
1	1885.80 (754.45)	162.22 (20.40)
2	2616.45 (811.09)	178.16 (95.17)
4	2101.87 (394.20)	183.01 (26.58)
8	1777.95 (1084.3)	210.76 (15.44)
12	744.48 (70.82)	365.80 (136.44)
24	600.76 (404.74)	124.56 (24.72)
48	149.91 (42.10)	65.41 (45.30)
72	121.84 (109.90)	15.75 (1.30)
96	64.64 (24.15)	11.62 (11.06)

Flumequine concentration (µg/kg) by HPLC		
Time (hours) post dosing	**Intramuscular [a]Mean (SD)**	**Oral dosing [a]Mean (SD)**
120	38.42 (19.15)	7.67 (6.18)
144	27.36 (11.90)	<5[b]
168	12.22 (3.29)	<5
192	6.66 (3.64)	<5
216	<5[b]	<5
240	<5	0
264	<5	0
288	0	0
312	0	0

a. pooled muscle tissue from 9 shrimp
b. limit of quantification 5 µg/kg

Figure 1. Mean muscle concentrations versus time profiles of flumequine in black tiger shrimp following single intramuscular and oral administration of 12 mg/kg



Medicated feed

Flumequine solution was mixed with pelleted feed to represent an oral dose equivalent to 12 mg/kg. The feed was ad libitum to the shrimp in the experiment for 5 consecutive days. Residue concentrations were determined daily for 15 days or until they fell under detection limit of 5 µg/kg. The results are presented in table 2. The maximum peak concentration was observed at day 3 of the treatment and decrease below the LOQ at 96 hours post-treatment. After intramuscular administration, the estimation half live (T1/2) was 33.4 hours, the relative bioavailability (F) after forced oral administration was 21.6% (table 3)

Table 2. Flumequine concentration in shrimp muscle following medicated feed application for 5 consecutive days at the dose of 12 mg / kg b.w

Time	Flumequine concentration (µg/kg) Mean (SD)
Day 1 of treatment	0
Day 2 of treatment	43.8 (15.2)
Day 3 of treatment	45.5 (14.1)
Day 4 of treatment	45.0 (9.17)
*Day 5 of treatment	29.8 (7.63)
24 hours	28.5 (14.5)
48 hours	22.7 (6.46)
72 hours	9.29 (5.73)
96 hours	<5
120 hours	<5
144 hours	0
168 hours	0

* Last day of medicated feed treatment

Table 3. Pharmacokinetic parameter for flumequine in shrimp

Parameters	Intramuscular	Oral dosing	Feed treatment
Dose (mg/kg shrimp or feed)	12	12	12
Water pH	8	8	8
$T_{1/2\beta}$ (h)	33.45	60.21	
MRT (h)	28.17	35.07	
AUC (µg.h^{-1}/kg)	56.55	12.23	
F_x %		21.63	
C_{max} (µg/kg)		365.81	45.52
T_{max} (h)		12.0	Day 3

$T_{1/2\beta}$: elimination half life; MRT: mean residence time; AUC: area under curve; F: availability of administered dose; Cmax: maximum tissue concentration; Tmax: time of peak tissue concentration

According the guidelines of Stamm (1989) concerning antibiotics used in aquaculture the plasma concentration of the drug should exceed its minimum inhibitory concentration (MIC) value by a factor of 3-4 times in the fish. The MIC of Aeromonas salmonicida for flumequine is below 0.063 mg/ml (Tsoumas et al., 1989). A single intramuscular injection of 12 mg/kg of flumequine maintained the flumequine above 250 µg/kg corresponding to 4 times the MIC values for 24 hours in shrimp. The maintained levels in excess of MIC value of 62.5 µg/kg are found for 48 hours in muscle, which the peak muscle concentration of 365 µg/kg at 12 hours after oral dosing. In comparison, medicated with flumequine in feed (12 mg/kg) for 5 consecutive days, the levels of drugs are low, only 30-45 µg/kg found in muscle which is below the MIC values at all sampling time. Therefore no drug efficacy using feed treatment at 12 mg/kg can be expected.

METHOD OF ANALYSIS

Samples of muscle tissues were homogenized following a modified procedure reported by Samuelsen (1990). The concentration of flumequine in muscle tissues were determined by high-performance liquid chromatography using a fluorescence detector set at excitation wavelength of 327 nm and emission wavelength of 369 nm (following Samuelsen and Ervik, 2001).

The method used ethyl acetate extraction. After evaporation the analyte was reconstituted in mobile phase. The mobile phase consisted of oxalic acid,acetonitrileand methanol. The separation was achieved by use of a reversed phase column in isocratic mode and the fluorescence detector was adjusted at 327 nm excitation and 369 nm emission.

Calibration curves were established in muscle tissues fortifying the samples to represent a range of 0.5-30 µg/kg and 100-200 µg/kg. The LOQ was 5 µg/kg and the curves were linear over the tested range. The linear correlation coefficients were 0.99968 and 0.99998, respectively. The recovery of flumequine was from 99.8% (2000 µg/kg) to 104.4% (5 µg/kg). The determination of flumequine metabolites was not carried out.

APPRAISAL

Considering the data available at the present meeting the Committee concluded that:

- The study showed that flumequine in an aqueous solution is relatively poorly absorbed by shrimp.
- Based on MRL of 500 µg/kg for muscle tissue in fish, and the results from the study using medicated feed at 12 mg/kg, flumequine concentration in tiger shrimps were below the tolerance all samples analyzed.
- The tissue residue depletion studies utilized methodology based on HPLC for separation and fluorescence detection. The LOQ, linearity and recoveries appeared acceptable.

Also the Committee concluded that they would justify establishing a MRL of 500 µg/kg in muscle in Black tiger shrimp provided the following information would be made available:

1. Detailed information on a regulatory method including method performance characteristics and method validation
2. Information on the approved dose for treatment of Black tiger shrimp and results of residue data from studies using the recommended dose.

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

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IMIDOCARB DIPROPIONATE

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ADDENDUM

To the Imidocarb Dipropionate residue monograph prepared by the 50th meeting of the Committee and published in FAO Food and Nutrition Paper 41/11, Rome 1999

The Committee in its review at the 50th Meeting requested the following information for evaluation in 2001:

- Depletion studies in non-lactating and lactating cattle using the recommended dose of imidocarb dipropionate for subcutaneous injection, with analysis of samples using the proposed regulatory method which includes the enzymatic digestion step, for comparison with the findings of the radiolabel study with respect to marker residue.
- A depletion study in sheep, using the recommended dose and mode of administration of imidocarb, for identification of marker residue and target tissues.

In response, the sponsor has provided additional information on an analytical method for the determination of imidocarb residues in bovine edible tissues and milk and applied the method in studies of the residue depletion of imidocarb in milk and edible tissues of cattle treated with the injectable product. The sponsor did not provide any additional residue data for sheep.

Residue Depletion Studies (with unlabelled drug)

Cattle

A depletion study was conducted under GLP in which 18 crossbred beef cattle (9 female, 9 male) each received a single subcutaneous injection of imidocarb dipropionate at the recommended therapeutic dose of 3.0 mg/kg bw (Nolan-Smith, 2001). The treated animals ranged in weight from 202 to 290 kg on the day prior to treatment and from 242 – 397 kg at slaughter. The controls, housed separately, weighed 200 – 272 kg on the day prior to treatment and 217 - 383 kg at slaughter. From the treated animals, eight of each sex were selected and 2 of each sex were killed at 30, 60, 90 and 180 days post-dosing. A pair of untreated control animals (1 female, 1 male) was killed with each of the 30-day and 180-day groups of treated animals in the depletion trial. Liver, kidneys, injection site and samples of muscle and fat were collected from each animal at slaughter and analyzed by a liquid chromatographic procedure which measures extractable residues after digestion with protease. Details of the method are given in a subsequent section of this report. Analytical results are reported as imidocarb free base, corrected for analytical recovery as determined from the intra-day assay data during validation. Recoveries were also calculated for each batch of samples analyzed, based on fortified blank tissues included with each batch.

Table 1. Imidocarb free base residues in tissues of cattle which received a single injection (SC) of imidocarb at 3.0 mg/kg bodyweight.[1]

Withdrawal time (days)	Mean recovery corrected imidocarb concentration (mg/kg)				
	Liver	Kidney	Muscle	Fat	Injection Site[2]
30	4.10 ± 0.87	13.94 ± 4.48	0.64 ± 0.09	0.09 ± 0.02	0.56 ± 0.25
60	1.36 ± 0.24	4.58 ± 1.35	0.27 ± 0.01	0.03 ± 0.01	0.28 ± 0.10
90	1.48 ± 0.62	3.71 ± 1.06	0.22 ± 0.04	0.02 ± 0.01	0.32 ± 0.06
180	0.39 ± 0.06[3]	0.92 ± 0.34	0.09 ± 0.04[4]	0.02 ± 0.01[5]	0.09 ± 0.04

[1] Performance characteristics of the analytical method for all edible tissues are given in Table 2.

[2] The mass of each injection site was adjusted to 300 g by multiplying the actual mass of the injection site by the recovery corrected concentration of imidocarb determined and dividing by 300.

[3] Mean of three results which were below the LOQ of 0.70 mg/kg; no residues were detected in the fourth sample, with an LOD of 0.20 mg/kg.

[4] Three of 4 results averaged were below the LOQ of 0.10 mg/kg, but above the LOD of 0.004 mg/kg; the fourth sample contained residues above the LOQ.

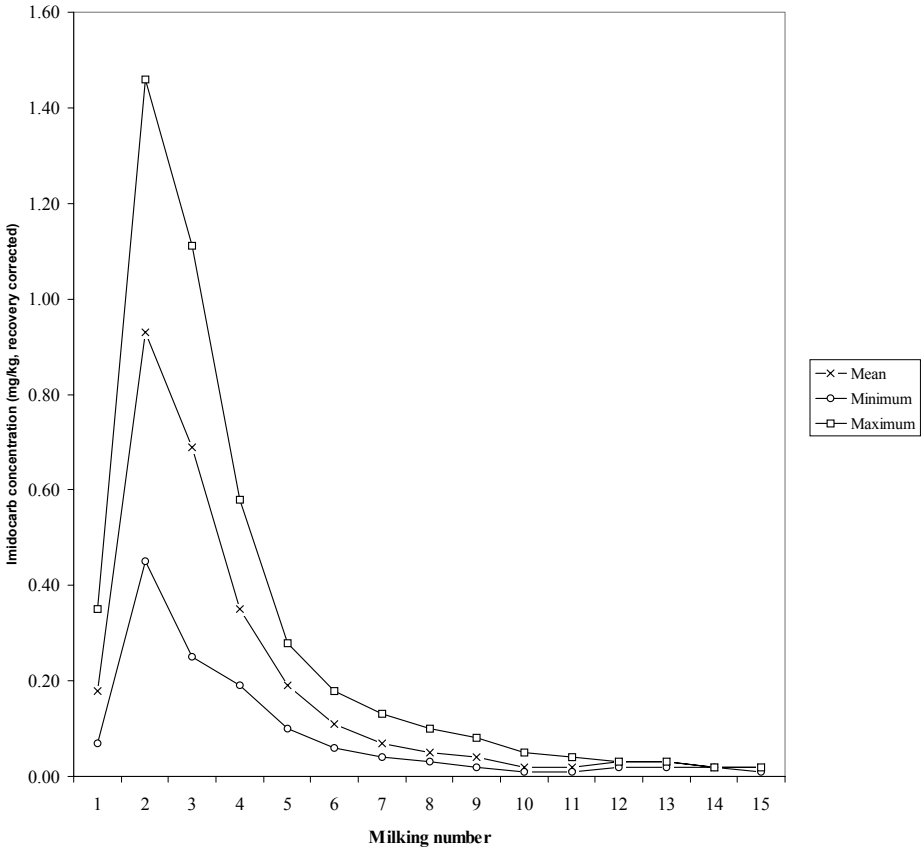
[5] Mean of three results, one of which was below the LOQ of 0.02 mg/kg; no residues were detected in the fourth sample, with an LOD of 0.006 mg/kg.

The results (Table 1) demonstrate that treatment with protease to release bound residues reveals a different residue distribution pattern in tissues, consistent with the results seen in the GLP study using ^{14}C-imidocarb dipropionate reviewed

by the 50th Meeting of the Committee. These results are therefore considered to more accurately reflect the results which would be determined in applying the proposed regulatory method to field samples and indicate that kidney is the preferred target tissue.

Lactating dairy cattle

Figure 1. Depletion of imidocarb free base residues in milk from cows which received a single SC injection of 3.0 mg/kg bw imidocarb dipropionate



In a GLP study, twenty-five lactating dairy cattle (504 – 750 kg at treatment) each received a single subcutaneous injection of imidocarb dipropionate at the recommended dose of 3.0 mg/kg bodyweight at approximately 5 hours after the morning milking (Nolan-Smith & Heal, 2001). The cows were categorized as low yielding (15 liters/day, or less – 8 cows), medium yielding (15 to 20 liters/day – 9 cows) or high yielding (20 or more liters/day – 8 cows). Two milkings were sampled prior to treatment (afternoon milking, Day "-1" and morning, Day "0"). The drug was administered after the morning milking on Day "0", then samples were collected at the afternoon milking on Day "0", at each of the two daily milkings (morning and afternoon, approximately 12-hour intervals) for 27 days, and at the morning milking on Day "28", for a total of 56 milkings following drug administration. Analyses were done using a liquid chromatographic method, described in the next section of this report. Samples were initially tested from one animal from each group for days 0 – 7 to provide a probable range for analysis of the other samples. Based on these results, only samples from days 0 – 5 were analyzed for the remaining animals (with one exception, where a day 6 sample was included as residues remained near the temporary MRL of 0.05 mg/kg in the day 5- PM sample). All results were reported as corrected for recovery, using the recovery of 88% determined during method validation.. As shown in Figure 1, all milk samples contained residues <0.05 mg/kg in the afternoon milking on Day 5. Analysis of samples of milk from the representative animals selected for the initial testing showed a continuing trend to concentrations near or below the limit of quantitation of 0.02 mg/kg by the Day 7 afternoon milking.

Methods of Analysis for Residues in Tissues and Milk

The method considered by the 50th Meeting of the Committee has been modified in a GLP study to remove some interference problems encountered with fat samples and to improve precision (Croucher & Dunn, 2001). Performance data were provided for the analysis of beef muscle, liver, kidney, fat and milk. Polypropylene tubes and silanized glassware are used throughout the procedure. After addition of dimethyl imidocarb internal standard, a 5-gram test portion of tissue (or 10 g of milk) is weighed into a 50 mL polypropylene tube. Recovery controls are also fortified at this point. 1M tri-(hydroxymethyl)methylamine buffer solution is added, then test portions are macerated and incubated with protease (from bovine pancreas). The incubated test portions are cooled to room temperature, acidified with hydrochoric acid and centrifuged. The decanted extract is made basic by addition of sodium hydroxide, then partitioned with hexane/isoamyl alcohol (3:2, v/v).

After removal of the hexane by evaporation, methanol and pH7-buffer are added and the extracts are cleaned up on a weak cation exchange (carboxylic acid) solid phase extraction cartridge. The collected fraction containing imidocarb and internal standard is reduced to dryness under a nitrogen stream and the final residue is dissolved in 1M hydrochloric acid. Liquid chromatographic analysis is conducted using a C18 reversed phase column with detection of imidocarb and the dimethyl imidocarb internal standard at 260 nm. The concentration of imidocarb is determined relative to the internal standard

response, using a calibration line based on the peak area of imidocarb relative to the internal standard over a range which includes the expected concentrations to be found in the samples. A linear response is expected.

The major numerical performance characteristics determined for the various matrices are summarized in Table 2. In addition, the method was demonstrated to have the required specificity in that imidocarb could be distinguished from other substances present in control samples and from a selection of commonly used veterinary drugs which might be present as incurred residues in field samples. Standard solutions of imidocarb and the internal standard, dimethyl imidocarb, prepared in 1M hydrochloric acid, were demonstrated to be stable in refrigerated storage (approximately 4°) over 40 days. Sample extracts were stable for 9 days under the same storage conditions, while milk and tissue samples stored frozen for 1 month at -20 ^{o}C showed no loss in residue concentrations. Additional data were provided to demonstrate that the method continues to meet required performance criteria at concentrations exceeding 4x the temporary MRLs recommended by the 50th Meeting of the Committee.

The limit of quantitation (LOQ) was established for each tissue based on a fitness for purpose approach, considering both the requirement that a regulatory method should be capable of quantitatively measuring residues at concentrations one-half the MRL and also the actual concentrations of residues found in tissues in the recent depletion studies. The LOQ was defined as the lowest concentration at which acceptable accuracy and precision could be demonstrated. In the case of the LOQs reported for imidocarb residues liver and kidney, there is a much larger difference from the limit of detection (LOD) than is usually reported.

Table 2. Performance characteristics of the liquid chromatographic assay for imidocarb residues in beef tissues and milk

Performance Characteristic	Liver	Kidney	Muscle	Fat	Milk
LOD (mg/kg)[1]	0.199	0.005	0.004	0.006	0.001
LOQ (mg/kg)[2]	0.702	0.526	0.105	0.018	0.018
Recovery (%)	87 (80-94)	86 (81-90)	84 (72-94)	86 (76-96)	88 (82-96)
Precision (%)	8.1	7.9	14.0	15.9	10.6

[1] Based on analysis of 20 controls, the mean of the measured content plus 3 standard deviations
[2] The lowest concentrations tested at which acceptable accuracy (recovery) and precision were obtained

MAXIMUM RESIDUE LIMITS

In recommending MRL's, the Committee may take into account the following factors:

- An ADI of 0-10 μg/kg bw was established by the Committee at its 50th 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 50th 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 50th meeting. The new data considered by the Committee at its present meeting confirm the concentrations predicted from the data for total residues in fat (reviewed by the Committee at its fiftieth meeting) using this factor. The present Committee rounded the percentages and assigned factors for correction of market-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 the 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 cows' milk.

Based on the above considerations, the following permanent MRLs were recommended by the Committee for edible tissues of cattle, expressed as imidocarb free base:

Muscle	300 μg/kg	Fat	50 μg/kg
Liver	1500 μg/kg	Milk (cows')	50 μg/kg
Kidney	2000 μg/kg		

The MRL's recommended above would result in a daily maximum intake of 523 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat and 1.5L of milk, as shown in Table 3.

Table 3. Theoretical Maximum Daily Intake of Imidocarb residues from beef and milk.

Tissue	Recommended MRL (µg/kg)	Food Factor (g)	MR/TR	Consumption (µg)
Muscle	300	300	0.9	100
Liver	1500	100	0.7	214
Kidney	2000	50	0.9	111
Fat	50	50	0.7	4
Milk	50 (µg/L)	1500	0.8	94
Total				523

APPRAISAL

The data provided by the sponsor address the requests from the 50th Meeting of the Committee for additional data on the depletion of residues in lactating and non-lactating cattle which have been treated with imidocarb dipropionate at the recommended dose and for analysis of the samples using the method with enzymatic digestion to release bound residues. Additional data were also provided to support modifications to the method reviewed by the 50th Meeting of the Committee.

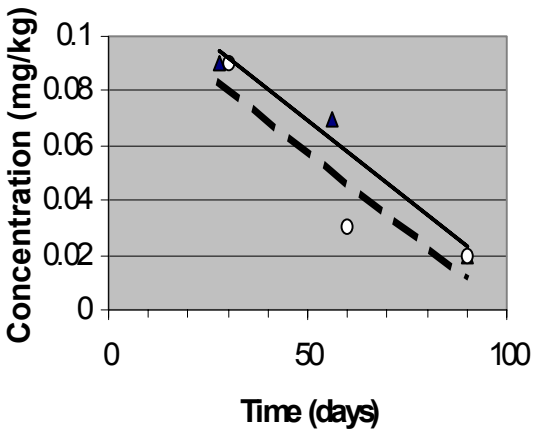
The depletion study in non-lactating cattle confirmed the general pattern of distribution of residues in various tissues shown in the earlier non-GLP studies reviewed by the 50th Meeting of the Committee. While differences in ages and weights of animals, treatment regimens, sampling times and analytical methodology make direct comparisons difficult, the data consistently demonstrate that the highest residues are found in kidney, followed by liver, muscle and fat. Liver was identified as the target tissue by the 50th Meeting of the Committee, based on results of earlier depletion studies which did not use the current procedures to release bound residues. However, the current GLP study clearly demonstrates that kidney is the preferred target tissue.

Residues are persistent. Most samples from any of the edible tissues contain detectable residues at 180 days after treatment at concentrations well below the temporary MRLs recommended by the 50th Meeting of the Committee, but all kidney samples and one liver sample at tested at 90 days post-treatment contained residues above these concentrations. The residues in kidney determined using the analysis with enzymatic digestion are higher than those reported previously, when the Committee established a higher temporary MRL for liver than for kidney. Based on the new data, the Committee considered it appropriate to adjust the MRLs to reflect the distribution of parent compound in these tissues, as reflected in the new GLP study in cattle.

As all residues in liver were below the LOQ of 0.70 mg/kg at day 180, the MRL for liver could be set at approximately 2x the LOQ, or 1.50 mg/kg. Similarly, the MRL for kidney could be adjusted to address the higher residue findings in the new study. Based on the data at Day 180, a concentration of 1.6 mg/kg could be suggested (mean + 2x std. dev.), or a "rounded up" value of 2.0 mg/kg could be proposed. This would, in effect, exchange the previous temporary MRLs for liver and kidney to reflect the actual distribution and would result a small reduction in the TMDI, as shown in Table 3.

The new data for analysis of residues in fat support the use of data from liver by the 50th Meeting of the Committee to identify a factor for correction of marker to total residues in fat. The analytical method for marker residue applied in the GLP study using ^{14}C-imidocarb propionate which that Committee used to establish factors was not able to detect marker residues in fat. Improvements to the method for application in the non-label study considered by the present Committee, however, revealed residues in fat consistent with the concentrations which would be expected if the factors were applied to the results from the earlier radiolabel study were calculated as marker residue only. The concentrations of total residue reported in the GLP study using ^{14}C-imidocarb propionate reviewed by the 50th Meeting of the Committee (Ferguson, 1996) were multiplied by the factor 0.68 to obtain predicted concentrations of the parent (marker residue). These were then plotted in comparison with the results reported in the depletion study provided for review by the present Committee (Nolan-Smith, 2001). The animals were administered equivalent amounts of the labelled and unlabelled drugs in the two respective studies, but there were small variations in the sampling times. As shown in Figure 2, the results reported in the new study with unlabelled drug agree with the predicted concentrations of parent compound calculated from the total residues reported in the earlier study. At 90 days, the predicted concentration from the radiolabel study matches the actual value determined in the new study with unlabelled drug. This substantiates the use of 0.68 as an appropriate factor for conversion of marker residue to total residue in the TMDI calculation.

Figure 2. Comparison of predicted marker residue concentration in fat calculated from radiolabel study (Ferguson, 1996) with marker residue concentrations measured in current study (Nolan-Smith, 2001)



The results of the depletion study in lactating dairy cattle follow a similar pattern to results in the earlier studies reviewed by the 50th Meeting of the Committee. The residues peak the day following treatment, then decline rapidly over the next 48 hours, after which there is a continued slow elimination at concentrations which are below the temporary MRL from Day 5 onwards in milk from cattle to which the drug has been administered. The data support a permanent MRL, with a note to national authorities that discard is advised for the next 8 milkings post-treatment. The data provided for treatment of dairy cattle at the therapeutic dose of 3.0 mg/kg bodyweight indicate that a minimum discard period of 96 hours (normally 8 milkings) is required for commingled milk from the herd, assuming one-third of the animals have been treated. Typically, therapeutic treatment would not include all animals in a herd. However, in situations where treatment of all animals in a herd with the therapeutic dose is required, a discard period of a minimum of 7 days appears prudent. The Committee considered, but did not recommend, that the discard period could be reduced in the normal circumstances of treatment of selected animals in a herd by 12 hours, or 1 milking, if the MRL for milk was established at 100 μg/kg. This would increase the TMDI to 617 μg, resulting in a theoretical daily consumption, which exceeds the ADI of 600 μg by 3%.

The sponsor has provided a suitably validated method to support the MRL for the marker residue, imidocarb free base. As noted in the previous discussion of the analytical method, the reported LODs for imidocarb residues in liver and kidney are considerably lower than the LOQs. In the case of these tissues, the reported LOQ could be better described as the "lowest calibrated level", or LCL. Laboratories wishing to apply the method to determine lower concentrations of imidocarb in liver or kidney may be able to validate the method performance at lower LOQs for these tissues. Typically, the LOQ is 2 – 3 times greater than the LOD, while in the reported validation, the LOQ for imidocarb residues is approximately 3.5 times the LOD for liver and 100 times the LOD for kidney.

The critical control points in the method, such as silylation of glassware and maintaining the SPE cartridges wet, have been identified. The recovery and precision meet the requirements for regulatory methods as established in Codex Alimentarius, Volume 3. Information on method specificity and analyte stability are provided. The method includes an internal standard, which corrects for recovery and validation data are provided to indicate that the method performs in a satisfactory manner at the temporary MRLs established at the 50th Meeting of the Committee, or at the proposed change in the recommended permanent MRLs considered by the present Committee. The method is suitable for use in a routine residue control laboratory.

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NEOMYCIN

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ADDENDUM

To the monographs prepared by the 43rd, 47th and 52nd meetings of the Committee published in the FAO Food and Nutrition Paper 41/7, Rome 1995, 41/9, Rome 1997 and 41/12, Rome 1999, respectively.

INTRODUCTION

The Committee has previously considered neomycin at the 43rd, 47th, 52nd and 58th meetings. The 43rd meeting of the Committee (WHO 1995) established a temporary ADI of 0–30 μg per kg bodyweight, based on the NOEL of 6 mg/kg bodyweight per day for ototoxicity in a 90-day study on the guinea pig and a safety factor of 200. The ADI was made temporary in view of deficiencies in the genotoxicity data. Gene mutation studies and an *in vivo* study on chromosome aberrations were requested for evaluation in 1996. Temporary MRLs of 5,000 μg/kg for kidney and 500 μg/kg for muscle, liver, and fat, expressed as the parent drug, were recommended for cattle, sheep, goats, pigs, turkeys, ducks and chickens. Temporary MRLs of 500 μg/kg and 500 μg/L, also expressed as the parent drug, were recommended for chickens' eggs and cows' milk, respectively.

The 47th meeting of the Committee (WHO 1998) considered new genotoxicity data for neomycin and, based on these data, established a full ADI of 0 - 60 μg/kg bodyweight, based on the NOEL of 6 mg/kg bodyweight per day for ototoxicity in the guinea pig and a safety factor of 100. Subsequently, the Committee recommended that the MRL for kidney for all species should be increased to 10,000 μg/kg. The higher MRL permitted the oral administration of neomycin sulphate, equivalent to 7.7 mg of neomycin base, per kg bodyweight per day on five consecutive days to non-ruminating calves. The Committee recommended also that the temporary status of all MRLs for neomycin be withdrawn.

The 52nd meeting of the Committee (WHO 2000) considered two new residue depletion studies. One study compared tissue residues following oral and intramuscular administration of neomycin to calves; the second study assessed tissue residue depletion in cattle after intramuscular administration of neomycin. The Committee concluded that although the MRLs for liver and kidney for cattle established at the 47th meeting were appropriate for oral formulations, the MRLs did not accommodate the use of injectable formulations of neomycin. Accordingly, MRLs for liver and kidney were increased to 15,000 μg/kg and 20,000 μg/kg, respectively, and MRLs of 500 μg/kg for muscle and fat, and 500 μg/L for milk were confirmed for cattle. The Committee confirmed also the MRLs of 10,000 μg/kg for kidney and 500 μg/kg for muscle, fat and liver for chickens, ducks, goats, pigs, sheep, and turkeys, and 500 μg/kg for chickens' eggs.

The 58th meeting of the Committee (WHO Technical Report Series, No 911, 2002) considered information on the registration of injectable neomycin products as well as how they were used in relation to good practice in the use of veterinary drugs. This followed a request by the Codex Committee on Residues of Veterinary Drugs in Foods at its 12th Session (ALINORM 01/31, paragraph 90). 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 authorised. The Committee also considered information about the toxicity of neomycin in calves and concluded that the information was relevant only to target animal welfare, which falls outside of the mandate of JECFA. Finally, the sponsor provided data in support of a proposal to increase the MRL of neomycin for milk, contending that an increase in the MRL was necessary to support practical withdrawal times for neomycin-containing intramammary products. In addition, the sponsor provided data to allow the MRLs for liver and kidney to be reconsidered. However, in the light of a request from the 13th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (ALINORM 03/31, paragraph 18) to evaluate new safety data, the Committee recommended maintaining the MRLs that had been recommended at the 43rd, 47th and 52nd meetings and deferring the review of the MRLs until such time as the toxicology of neomycin was re-evaluated.

The 60th meeting of the Committee evaluated new safety data, which comprised information on microbiological aspects of consumer safety of neomycin and the evidence for a link between the presence of a specific mutation to mitochondrial DNA in humans and increased susceptibility to aminoglycoside ototoxicity. The Committee confirmed the ADI of 0 – 60 μg per kg bodyweight, based on the NOEL of 6 mg/kg bodyweight per day for ototoxicity in a 90-day study on the guinea pig and a safety factor of 100. The 60th meeting of the Committee also evaluated the residues depletion data submitted by the sponsor to the 58th meeting.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Neomycin is an aminoglycoside antibiotic produced by *Streptomyces fradiae* and is a mixture of neomycin A (<1% present in commercial preparations), neomycin B (>90% present in commercial preparations) and neomycin C. Other aminoglycoside antibiotics include streptomycin, kanamycin, gentamicin, tobramycin and amikacin. Aminoglycosides enter susceptible bacteria by oxygen-dependent active transport and by passive diffusion, and then bind irreversibly to the 30S bacterial ribosomes (Brown, 1988). This blocks the formation of a complex that includes mRNA, formylmethionine, and tRNA, and induces the misreading of the genetic code on the mRNA template. As a result, the tRNA is translated incorrectly, producing a non-functional protein. Aminoglycosides have additional effects on microorganisms such as interference with the cellular electron transport system, induction of RNA breakdown, inhibition of translation, effects on DNA metabolism, and damage to cell membranes. The bactericidal effect is through the formation of abnormal cell membrane channels by misread proteins (Prescott, Baggot and Walker, 2000).

Aminoglycosides are utilized primarily in the treatment of infections caused by aerobic Gram-negative microorganisms. They can be effective in the treatment of some Gram-positive organisms such as *Staphylococcus aureus*, some mycobacteria, some Mycoplasma strains, and some spirochetes. Aminoglycosides are not active against anaerobic organisms. Neomycin demonstrates bactericidal activity against most Gram-positive and Gram-negative rods, many Gram-positive cocci, and such acid-fast pathogens as *Mycobacterium tuberculosis*. Acidic or purulent conditions at the site of infection can limit the efficacy of aminoglycosides, as can the presence of cations (Prescott, Baggot and Walker, 2000).

Neomycin is formulated, either alone or in combination with other antimicrobials such as lincomycin, penicillin, cephalosporins and some sulphonamides, for oral (including in-feed and medicated drinking water) administration and for injection, intramammary infusion and topical (including ocular) application. In aquaculture, neomycin is administered as a bath solution.

Neomycin has a long history of use. It is indicated in the treatment of intestinal and respiratory infections, wound and skin infections, and mastitis (Prescott, Baggot and Walker, 2000). Orally it is used to treat enteric infections, including salmonellosis and enterotoxigenic *Escherichia coli* diarrhoea in calves. Neomycin is also administered to cattle by intramuscular injection to treat respiratory tract infections, and by intramammary infusion, most commonly in combination with other antibiotics, to treat mastitis in lactating and non-lactating dairy cows. Other clinical applications of neomycin include intrauterine administration for uterine infections, the oral treatment of pigs with coliform diarrhoea, and of chickens and turkeys with salmonellosis, and topical administration of infectious conditions of the eye and external ear, as well as in contaminated wounds. Neomycin is administered by intramuscular or intravenous injection to foals with *Rhodococcus equi* pneumonia.

Because the use of injectable formulations of neomycin is associated with ototoxicity (deafness in cattle) and nephrotoxicity, including at doses indicated, such use is generally limited to the treatment of serious Gram-negative infections resistant to less toxic medications or as an alternative to costly medications . In some countries such as the USA, Canada and South Africa, injectable neomycin products are not authorised for use in food animals on account of such use being associated with a high risk of toxicity.

Dosage

Table 1. Maximum daily doses of neomycin reported in studies submitted to the 43rd, 47th, 52nd and 58th meetings of JECFA

Species	Oral	Parenteral	Intramammary
Poultry	20 mg/kg		
Pigs	15 mg/kg		
Cattle	15 mg/kg	12 mg/kg intramuscular	
Sheep	15 mg/kg		
Goats	15 mg/kg		
Lactating dairy cows			100 mg/quarter every 12 hours

METABOLISM

Pharmacokinetics

General

The pharmacokinetic properties of neomycin are largely attributed to it being a polar organic base (Prescott, Baggot and Walker, 2000). Neomycin is generally not significantly absorbed from the gastrointestinal tract, with very young calves being an exception. Aschbacher and Feil (1994) reported that 11 % of an oral neomycin dose of 30 mg/kg bodyweight was absorbed in 3-day-old calves and 1 to 2 % of the dose was absorbed by 2-month-old calves, regardless of the status of ruminal development. Damage to the gastrointestinal mucosa can also lead to increased aminoglycoside absorption (Thomson et al, 1991; Brown and Riviere, 1991). The binding of neomycin (at concentrations of 5 – 10 μg/mL) to plasma proteins is reportedly 45 % in cows and 50 % in ewes (Ziv and Sulman, 1972). The poor diffusion of neomycin across biological membranes can be attributed to its poor lipid solubility. Selective binding to tissues, including kidney cortex, occurs, resulting in residues that persist in animals for prolonged periods. As a result, a dose-dependent, slow elimination phase (gamma-phase), many times longer than the initial elimination phase has been described (Brown et al, 1985). The aminoglycosides, as a class, undergo negligible metabolism after parenteral administration (Bevan and Thompson, 1983). Neomycin is excreted in the faeces after oral doses and in the urine (glomerular filtration) after parenteral administration (Prescott, Baggot and Walker, 2000; WHO 1995).

Lactating dairy cattle

Eight healthy lactating dairy cows received intramammary infusions of Lincocin Forte® Sterile containing 100 mg neomycin base and 330 mg lincomycin base into each mammary quarter at 12-hour intervals following three successive milkings (Deluyker et al, 1996). Heparinised blood samples were collected prior to treatment and at 0.5, 1, 2, 4, 8, 12, 24 and 36 hours after the first infusion. Plasma separated from the blood samples was assayed for neomycin using solid phase extraction and HPLC. Neomycin was not detected (<0.024 μg/mL) in any of the plasma samples. Milk excretion of neomycin estimated from the total amount of neomycin recovered in pooled milk up to 120 hours post-treatment, based on measured milk production, was 55.7 ± 9 % of the total dose administered.

MILK RESIDUE DEPLETION STUDIES

The Committee evaluated one new residues depletion study in milk, in which unlabelled neomycin was administered by intramammary infusion to lactating dairy cows. Eight cows were common to the pharmacokinetic study (see previous section). The milk residue study was GLP-compliant.

Lactating dairy cattle

Twenty-four healthy lactating cows were assigned to four blocks of 6 cows each according to parity and milk yield. Two lactating cows from each block were randomly re-assigned to a pharmacokinetic group (n = 8). All 24 animals were used in the milk residue depletion study. The cows all received intramammary infusions of Lincocin Forte® Sterile containing 100 mg neomycin base and 330 mg lincomycin base in each mammary quarter at 12-hour intervals after three successive milkings (Deluyker et al, 1996).

In the pharmacokinetic group of cows, quarter milk samples were collected before each infusion and continued until the second milking after the last infusion. Quarter milk production, starting from 6 milkings before treatment up to and including 10 milkings after the last infusion, was also measured. Milk samples were assayed for neomycin concentration using solid phase extraction and HPLC. Neomycin was not detected (<0.0327 μg/mL) in milk prior to the first infusion, whereas the mean neomycin concentration in milk collected before the second and third infusions was 22.2 μg/mL and 29.7 μg/mL, respectively. At 12 hours and 24 hours after the last infusion, the mean neomycin concentrations were 28.0 μg/mL and 4.92 μg/mL, respectively.

The depletion of neomycin residues in milk from all 24 cows, including the pharmacokinetic group, was determined from pooled milk samples collected before every infusion and until the tenth milking after the last infusion. Pooled milk samples were assayed for neomycin concentration using solid phase extraction and HPLC. The LOQ of the analytical method was 0.1 μg/mL. The results for the individual cows are shown in Table 2. The mean neomycin concentrations at 12 and 24 hours after the last infusion were 24 μg/mL and 4.8 μg/mL, respectively. At 60, 72 and 84 hours after the last infusion, the mean (range) neomycin concentrations in pooled milk samples were estimated to be 0.26 (<LOQ – 1.05), 0.21 (<LOQ – 0.65) and 0.16 (<LOQ – 0.51) μg/mL, respectively. Statistical tolerance limits for the neomycin milk residue concentration versus time depletion curve were determined by linear regression of the logarithmic concentrations of neomycin in milk versus time, and then estimating the upper one-sided 95% confidence interval for the 95th percentile of a population receiving the described treatment. Upper limits of 1,800 μg/kg, 1,500 μg/kg and 1,000 μg/kg were determined for neomycin concentrations in milk samples at 72 hours, 76 hours and 84 hours, respectively.

Table 2. Neomycin residues (μg/mL) in milk after three successive intramammary infusions of 100 mg neomycin base into each quarter of the udder at 12-hour intervals

Cow No	Hours after last infusion									
	12	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**
4	8.67	3.14	1.17	0.49	0.15	0.11	0.12	<LOQ	<LOQ	<LOQ
9	18.0	8.78	3.74	1.59	0.63	0.48	0.17	0.13	0.15	<LOQ
11	17.4	4.85	1.14	0.42	0.16	0.12	0.12	<LOQ	<LOQ	<LOQ
16	20.3	2.03	0.40	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
18	27.2	4.82	0.74	0.14	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22	20.3	3.36	2.67	1.49	0.37	0.45	0.51	0.18	0.26	0.19
27	20.8	3.64	1.41	0.52	0.20	0.13	<LOQ	<LOQ	<LOQ	<LOQ
28	20.3	3.90	0.75	0.27	0.10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
31	29.4	6.30	1.10	0.28	0.15	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
37	16.0	2.96	0.94	0.25	0.12	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
40	15.3	1.63	0.94	0.36	<LOQ	0.11	<LOQ	<LOQ	<LOQ	<LOQ
41	29.9	3.41	0.43	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
46	18.9	6.15	5.51	1.78	0.76	0.65	0.30	0.52	0.48	0.11
47	34.7	13.7	7.12	2.06	0.56	0.55	0.21	0.14	0.12	<LOQ
48	7.71	1.59	0.28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
50	16.7	5.23	2.67	0.81	0.26	0.24	0.19	0.084	<LOQ	<LOQ
53	25.2	2.36	0.43	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
57	33.7	4.41	1.63	0.57	0.17	0.21	0.18	<LOQ	<LOQ	<LOQ
60	44.6	6.19	1.59	0.35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
61	19.5	3.53	1.26	0.48	0.11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
63	30.7	6.58	2.70	0.86	0.46	0.22	<LOQ	<LOQ	<LOQ	<LOQ
65	20.6	3.72	1.54	0.44	0.20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
71	49.5	9.97	6.49	1.46	1.05	0.58	0.47	0.24	0.20	0.16
73	31.3	2.88	0.65	0.10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mean	24.0	4.80	1.97	0.63	0.26	0.21	0.16			
SD	10.1	2.83	1.92	0.59	0.25	0.18	0.11			
Min.	7.71	1.59	0.28	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Max.	49.5	13.7	7.12	2.06	1.05	0.65	0.51	0.52	0.48	0.19

LOQ = 0.1 μg/mL in milk

MILK PRODUCTION AND SOMATIC CELL COUNTS

Milk production and somatic cell counts were monitored prior to and throughout the milk residue depletion study. The milk production data confirmed that low and high yielding cows had been selected for the trial. Milk production was not affected by treatment and remained at pre-treatment levels for at least 84 hours after the last infusion. Decreased milk production was reported at 96 hours or later following the last infusion for cows 31, 46, 53 and 73. Somatic cell counts remained at pre-treatment levels throughout both the infusion and post-infusion periods, except for cows 31 and 46 which had elevated somatic cell counts commencing at 72 hours after the last infusion. The cows with decreased milk production and/or increased somatic cell counts were investigated further, and shown to have acquired yeast mastitis (*Candida kefir*) unrelated to the intramammary formulation.

EFFECT OF NEOMYCIN ON STARTER CULTURES IN MILK PROCESSING

The effect of neomycin on bacterial starter cultures used in the production of Italian cheese, yogurt, buttermilk and sour cream was assessed based on "time to clot" ratios (Hallberg et al, 1994). Neomycin concentrations in milk of less than 2 μg/mL were shown to have no effect on the growth of the bacteria in any of the starter cultures.

TISSUE RESIDUE DEPLETION STUDIES

The Committee evaluated one GLP-compliant tissue residue depletion study, which involved the oral administration of neomycin to calves. An additional study of tissue residues in heifers, which did not comply with Good Laboratory Practice,

was not considered as the animals received less than half the total dose administered in the first study and less than the dose recommended by the sponsor.

Calves

Sixteen non-ruminating Holstein bull calves of approximately 35 kg bodyweight were treated orally for 14 consecutive days with neomycin sulphate, equivalent to 15 mg of neomycin base, per kg bodyweight (Arnold et al, 1991). Groups of four calves were sacrificed at 7, 14, 21, or 28 days after the last treatment. Livers and kidneys were analysed for neomycin residues using a microbiological assay with a LOQ of 0.92 μg/g (Stahl, 1991). The bacterial test strain was *Staphylococcus epidermidis* UC 719 (Official Method of Analysis, 1984). The results are presented in Table 3.

Table 3. Neomycin residues (μg/g) in the kidneys and livers of calves

Animal No.	Days after last treatment	Kidney	Liver
150	7	71.3	2.5
158	7	55.3	<LOQ
160	7	30.4	2.16
168	7	36.8	2.18
149	14	10.0	<LOQ
161	14	7.3	<LOQ
163	14	10.6	<LOQ
167	14	8.4	<LOQ
156	21	11.5	<LOQ
157	21	13.1	2.22
164	21	3.5	<LOQ
166	21	5.0	<LOQ
152	28	6.8	<LOQ
159	28	3.9	<LOQ
162	28	4.6	<LOQ
165	28	5.0	1.17

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. These liver data were not validated by data on stability during 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 2,200 μg/kg and 1,200 μg/kg were found, respectively. Due to the paucity and quality of the data for both kidney and liver, no conclusions were drawn from the study.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Chemical Methods

The 43rd meeting of the Committee considered an HPLC method of analysis for neomycin residues in milk with a limit of detection of 0.050 μg/g (Agarwal, 1990).

A method reported by Deluyker et al (1996) for the quantification of neomycin in milk of cattle was considered by the present Committee. A trichloroacetic acid extract of milk was prepared, neutralised with sodium sulphate/sodium monohydrogen phosphate and centrifuged. The supernatant fraction was retained, adjusted to pH 6.8 with NaOH, and applied to a carboxylic acid cartridge that had been preconditioned sequentially with methanol and 0.1 M Na_2SO_4/Na_2HPO_4, pH 6.8. Following cartridge clean-up using water, the neomycin residue was eluted with citrate/phosphate buffer pH 2.3.

HPLC determination of neomycin was performed on a C_{18} column with post-column derivatisation with o-phthalaldehyde and fluorescence detection (340 nm excitation, 435 nm emission). Under these conditions, neomycin chromatographed at approximately 12 minutes.

Validation in bovine milk

Accuracy of the method was evaluated with fortified quality control (QC) samples at concentrations of 0.100 and 0.103 μg/mL (QC-low samples), and 4.008 and 4.115 μg/mL (QC-high samples). Results ranged from 95.3 – 95.9 % for QC-low samples and from 102 – 103 % for QC-high samples. The acceptance range for QC-low samples was 80 – 120 % and for QC-high samples was 80 – 115 %. Runs were accepted if more than 75 % of the determined QC sample concentrations were within the acceptance range.

Recovery determinations were conducted at fortification concentrations of 0.1, 0.25, 0.5, 1, 2 and 5 μg/mL in blank solid phase extracts. The mean recoveries at the respective concentrations were 56.5, 53.3, 59.5, 60.7, 60.2 and 60.4 %; the overall recovery (mean ± SD) was 58.7 ± 4.4 % (n = 16).

Linearity of calibration curves (r > 0.9962) was demonstrated for concentrations of neomycin between 0.05 and 5 μg/mL.

Limit of detection (LOD) for the method was defined as the mean plus three standard deviations of noise concentration in blank chromatograms at the location where neomycin eluted. In this study, blank milk samples from 24 cows were prepared, extracted and chromatographed, and the LOD for the method was calculated to be 0.03 μg/mL.

Limit of quantitation (LOQ) for the method was defined as the lowest concentration that can be determined with a precision (CV %) of <20 %. Milk was fortified, prepared, extracted and nine replicates chromatographed over three runs. The LOQ for the method was established as 0.1 μg/mL with a precision of 12.7 %.

Repeatability, expressed as the mean intra-day precision (CV %), was 15.9 % (n = 14) and 6.7 % (n = 6) for QC-low samples, 6.5 % (n = 14) and 4.6 % (n = 6) for QC-high samples, and 4.7 % (n = 22) for replicate analyses of incurred samples.

Reproducibility, expressed as the mean between-day precision (CV %), was 0.0 % (n = 14) and 8.1 % (n = 6) for the QC-low samples, 3.0 % (n = 14) and 3.9 % (n = 6) for QC-high samples, and 7.7 % (n = 17) for replicate analyses of incurred samples.

Specificity from matrix components and from lincomycin (at a concentration of 8.92 μg/mL) was assessed and no interference was demonstrated in either situation. Specificity of the method in the presence of other veterinary drugs was not described.

Stability of neomycin in milk during storage was investigated. Milk was fortified at 46.1 μg/mL and stored at ≤-20°C for 163 days or 210 days. These storage times exceed the maximum storage time for the experimental milk samples of 119 days. The mean neomycin concentrations were 103 % and 135 % of the fortified levels following storage at 163 days and 210 days, respectively. Overall, the stability of neomycin in milk upon storage is acceptable. It is noted, however, that when fortified milk samples were stored for 210 days, recoveries significantly exceeded 100 %. The sponsor suggested that an incorrect dilution might have explained this high value.

Microbiological Methods

Quarter and pooled milk samples were collected from eight cows immediately prior to the second and third intramammary infusions and assayed for neomycin by a microbiological method and HPLC. The bacterial test strain used in the microbiological assay was *Escherichia coli*. It was claimed that the microbiological assay had been validated in study 94.087.3 (a copy of which was not provided) and only limited validation data were provided in the present submission. The latter did not include either the LOD or the LOQ for the method but reported the recoveries from fortified milk to be 89 % and 88 % at 2.51 μg/mL and 101 μg/mL, respectively.

Comparison of Neomycin Concentrations in Milk Measured by HPLC and the Microbiological Method

Concentrations of neomycin determined by HPLC and a microbiological method in selected quarter and pooled milk samples were compared. Good agreement was demonstrated (Table 4).

Table 4. Comparison of neomycin residues (μg/mL) in milk measured by HPLC and a microbiological method (MB).

	Before the second infusion				Before the third infusion			
Cow No.	Quarter milk		Pooled milk		Quarter milk		Pooled milk	
	HPLC	MB	HPLC	MB	HPLC	MB	HPLC	MB
16	41.8	39.1	14.2	9.79	57.5	29.4	14.9	10.0
	41.1	32.7			53.9	27.1		
	23.9	21.2			36.5	11.7		
	28.7	21.7			39.7	24.4		
18	52.2	65.7	23.2	21.9	54.3	51.2	30.1	13.4
	41.8	41.9			42.1	47.6		
	33.5	29.4			48.7	46.6		
	36.3	31.6			52.5	29.1		
22	30.6	36.0	12.5	18.2	32.4	34.8	15.4	13.7
	31.2	33.1			43.9	48.3		
	15.0	17.3			21.8	22.9		
	18.8	18.8			15.3	18.5		

	Before the second infusion				Before the third infusion			
Cow No.	Quarter milk		Pooled milk		Quarter milk		Pooled milk	
	HPLC	MB	HPLC	MB	HPLC	MB	HPLC	MB
27	27.1	25.4	12.8	12.4	50.7	46.1	13.7	9.79
	24.7	26.0			46.4	21.6		
	16.5	19.7			30.4	25.1		
	18.0	14.7			27.3	20.4		
28	38.8	32.5	15.0	17.4	25.2	26.0	18.4	12.1
	9.19	23.4			36.0	38.2		
	23.5	26.3			27.3	21.8		
	41.0	40.1			34.4	32.9		
37	7.58	9.65	7.85	6.13	14.8	11.1	10.4	12.4
	3.19	5.07			10.5	9.77		
	7.70	7.82			15.3	31.9		
	18.6	20.0			25.4	29.4		
46	11.6	19.0	8.80	14.6	12.9	15.6	16.4	35.0
	8.72	15.6			14.3	19.7		
	11.7	16.0			16.2	19.0		
	5.13	15.1			13.0	15.8		
50	13.7	16.7	7.68	11.3	17.3	20.4	9.90	8.51
	13.1	13.1			13.2	16.0		
	8.55	12.8			11.4	11.0		
	7.34	10.2			16.8	14.4		

Qualitative tests for milk

A report by Deluyker et al (1996) that investigated the suitability of five qualitative tests for detecting neomycin residues at concentrations approximating the milk MRL was assessed. The tests considered were Delvotest® SP, Penzym, Valio T101, Brilliant Black Reduction, and Dutch Tube Diffusion. Blank milk samples fortified with neomycin were used to establish the detection levels of each of the five tests. The results are shown in Table 5.

Table 5. Detection levels of neomycin (μg/mL) in milk for qualitative screening tests

Qualitative Screening Test	Neomycin (μg/mL)
Delvotest® SP	0.20
Penzym	> 2.0
Valio T101	> 2.0
Brilliant Black Reduction	2.0
Dutch Tube Diffusion	0.10

Pooled milk samples from twenty-four dairy cows involved in the residues depletion trial (Deluyker et al, 1996) taken from 60 hours to 120 hours after the third infusion of Lincocin Forte® Sterile were subjected to the five qualitative tests. The results are presented in Table 6.

Notwithstanding the non-specific nature of qualitative tests, the results generally reflect the status of neomycin residues in milk since data provided by the sponsor demonstrated that residue depletion for neomycin was slower than for lincomycin following intramammary infusion with Lincocin Forte® Sterile. Large variations in the duration and number of positive tests were observed, however, no positive results occurred with the Brilliant Black Reduction test in samples taken 84 hours after the last infusion or later. False-positive results for mastitic milk were observed with the Penzym, Valio T101, and Dutch Tube Diffusion tests.

Table 6. Number of negative (N), uncertain (U), or positive (P) results for qualitative tests on milk

Test	Hours after last infusion						
	Result	60	72	84	96	108	120
Delvotest® SP	N	7	8	11	16	19	21
	U	9	9	9	5	4	2
	P	8	7	4	3	1	1

Test	Hours after last infusion						
	Result	60	72	84	96	108	120
Penzym test	N	18	14	18	18	19	21
	U	6	9	6	6	5	2
	P	0	1	0	0	0	1
Valio T101 test	N	6	17	24	24	23	23
	U	0	0	0	0	0	0
	P	18	7	0	0	1	1
Brilliant Black Reduction test	N	12	18	20	23	24	23
	U	10	5	4	1	0	1
	P	2	1	0	0	0	0
Dutch Tube Diffusion test	N	6	6	7	9	11	16
	U	7	8	11	9	10	4
	P	11	10	6	6	3	4

APPRAISAL

The clinical pharmacology of the aminoglycosides has been reviewed recently by Prescott, Baggot and Walker (2000). Neomycin is used for the local treatment of intestinal infections, of wound or skin infections, and of mastitis. For these clinical conditions, neomycin is formulated either alone or in combination with other antibiotics for oral, topical or intramammary administration. The toxic side effects of neomycin are generally not evident following such use. By contrast, the systemic use of neomycin is limited by a relatively high risk of nephrotoxicity and ototoxicity (deafness). Neomycin is considered the most nephrotoxic aminoglycoside (Riviere, Craigmill and Sundlof, 1991). Aminoglycosides cause nephrotoxicity by accumulating in the proximal tubular cells, where they interfere with cellular metabolism and transport processes. The initial tubular changes can progress to proximal tubular necrosis, followed by perturbations in glomerular filtration, and azotemia. The auditory ototoxicity associated with the systemic use of neomycin may be due to the drug's distribution characteristics and its ability to accumulate in the cochlear, causing severe cochlear toxicity (Kitasato et al, 1990). Vestibular, in addition to auditory, ototoxicity can occur with parenteral neomycin, but damage of cranial nerve VIII is usually not seen unless parenteral therapy is extended past 5 days (Bowen and Crawford, 1976). The neuromuscular blocking effects of neomycin that have been demonstrated during pentobarbital anaesthesia in nonhuman primates (Adams, 1973) are considered rare compared to its nephrotoxic and ototoxic effects. Because of these toxicity concerns, neomycin is not recommended for systemic use on animals. Importantly, alternate drugs that are safer and demonstrate equal or better efficacy than neomycin are readily available for parenteral use. Despite this, neomycin is approved for parenteral administration to food animals in some countries, and is regarded as an inexpensive "alternative" to gentamicin.

In respect to good practice in the use of veterinary drugs, injectable formulations of aminoglycosides are generally dosed to achieve a high peak blood concentration (typically 8 to 10 times higher than the minimum inhibitory concentration for the microorganism), followed by a low trough concentration, significantly below therapeutic blood concentrations. This strategy is justified since aminoglycosides kill bacteria by a concentration-dependent mechanism (Campbell et al, 1996), with the length of time the organism is exposed to the antibiotic being of lesser importance (Xiong et al, 1997). Because the occurrence of nephrotoxicity due to aminoglycosides is more influenced by the trough than the peak blood concentrations, a dosing strategy is applied whereby the interval between treatment is extended to ensure that the trough drug concentrations drops low enough, a concentration specific for each aminoglycoside, to minimise toxicity. This phenomenon has been extensively studied with gentamicin (Cummings et al, 1990; Grauer, 1996) and to some extent with amikacin (Brown and Riviere, 1991). By comparison, scant information on this approach is available for neomycin, most likely due to its toxicity at therapeutic dose rates, which is well documented.

Presently, the available information pertaining to the registration of injectable neomycin products and how they are used with respect to Good Veterinary Practices is incomplete. Injectable neomycin products are not approved for use in food animals in the USA, Canada or South Africa whereas a very small number of such products are approved in Australia, the Czech Republic and Thailand. From the preceding discussion, it would appear that: (i) compelling evidence exists on the toxicity of neomycin when administered by parenteral injection; (ii) an approach to parenteral dosing with neomycin that both provides therapeutic concentrations and overcomes toxicity concerns has not been proposed; and (iii) safe alternatives, with equal or better efficacy than neomycin, are approved for parenteral administration to food animals.

From a residues perspective, neomycin residues are characterised by persistence in kidney, and to a lesser degree in liver and at injection sites. The bioavailability of neomycin markedly influences the magnitude of the incurred residues and, in turn, the time required for residues to deplete. In calves, for example, the bioavailability of oral doses of neomycin ranges from 1 to 11 %, depending on the age of the calves (Aschbacher and Feil, 1994). In this respect, it was noted by the 47th meeting of the Committee that increasing the (temporary) MRL for kidney from 5,000 μg/kg to 10,000 μg/kg permitted the practical use of formulations administered orally to very young calves. It should be noted that neomycin is not approved for use in veal calves in some countries; this overcomes the concerns relating to the occurrence of neomycin residues in very young calves. The 52nd

meeting of the Committee noted that its recommendations to increase the MRL for kidney from 10,000 μg/kg to 20,000 μg/kg, and to increase the MRL for liver from 500 μg/kg to 15,000 μg/kg, allowed practical withdrawal times to be established for injectable formulations of neomycin. The latter reflects the fact that neomycin is readily bioavailable when injected.

One study in the literature of particular relevance to the injectable use of neomycin investigated both neomycin toxicity and kidney residues in four heifer calves weighing 150 – 190 kg bodyweight (Crowell et al, 1981). Results from the study are shown in Table 7 and indicate that toxic manifestations occur as early as 5 days after the initiation of parenteral dosing regimens. The study demonstrates that nephrotoxicity and deafness in cattle occur at sub-maximal dose rates. It was noted also that these toxic effects occurred when residue concentrations in the kidneys were less than 500 μg/kg.

Table 7. Neomycin toxicity and kidney residues in calves that were administered neomycin by intramuscular injection.

Calf No.	Neomycin Treatment Regimen	Days after first neomycin injection when symptom first occurred			Days after the last injection	Kidney Residues (μg/kg)
		Renal casts	Azotemia	Deafness		
1	**4.5 mg/kg IM twice daily for 12 days**	5	10	None*	0.25	300
2	**4.5 mg/kg IM twice daily for 12 days**	5	12	14	1	226
3	**2.25 mg/kg IM twice daily for 13 days**	12	12	19	6	210
4	**2.25 mg/kg IM twice daily for 13 days**	10	12	None	11	430

* Calf 1 was euthanised at 12 days after the first injection of neomycin; it may have become deaf had it survived longer.

The data suggest that the use of injectable formulations of neomycin in food animals does not represent good practice in the use of veterinary drugs, and that injectable neomycin formulations should be excluded from consideration when recommending MRLs. The sponsor who submitted data in support of the injectable uses of neomycin has confirmed that they do not wish to defend the injectable use patterns. Furthermore, information on the registered use patterns for injectable formulations of neomycin in food-producing animals provided by Member Governments in response to a request of the 12th Session of CCRVDF indicated that use of parenteral formulations is not regarded as good practice in the use of veterinary drugs, and few such products were approved. The MRLs for kidney of 20,000 μg/kg and for liver of 15,000 μg/kg recommended by the 52nd meeting of the Committee were therefore considered by the present Committee to be unnecessary.

One GLP-compliant milk residue depletion study, which used unlabelled compound, was considered. The recommended label rate of 100 mg of neomycin base was infused into each quarter of the udder at 12-hour intervals following three successive milkings. The formulation was well tolerated. Unrelated to the formulation was the development of yeast mastitis in four of twenty-four cows; the causative microorganism was *Candida kefir*. The four cases of mastitis occurred late in the study and did not compromise the findings.

Toxicity associated with systemic uptake of neomycin following the intramammary infusion of Lincocin Forte® Sterile was not manifested in the studies considered by the Committee. Indeed, neomycin was not detected (<0.024 μg/mL) in any of the plasma samples in the pharmacokinetic study on lactating dairy cows that received intramammary infusions of Lincocin Forte® Sterile into each mammary quarter at 12-hour intervals following three successive milkings. Although the study does not provide supporting evidence of systemic uptake of neomycin following intramammary infusion, neither does it rule out the possibility of some systemic absorption occurring. For example, the average daily intramammary dose administered to the lactating cows in the pharmacokinetic study was 0.65 mg/kg, considerably less than the recommended oral and parenteral doses of neomycin, which may explain why neomycin could not be detected in plasma even in the presence of intramammary absorption. The recovery of neomycin in pooled milk up to 120 hours post-treatment based on measured milk production was 55.7 ± 9 % (mean ± SD) of the total dose administered which may suggest that some absorption could have occurred. In a study reported by the EMEA (2000), absorption following intramammary administration of neomycin was confirmed in 16 healthy cows that received an intramammary infusion containing 300 mg lincomycin and 100 mg neomycin base, as neomycin sulphate, in each of 4 udder quarters, following each of 3 successive milkings at 12 hour intervals. In that study, measured concentrations of neomycin residues were only present in the kidney and udder. For the kidney, mean concentrations were 700 μg/kg (day 1), 315 μg/kg (day 7), and 205 μg/kg (day 14). The mean concentrations were below the limit of quantification (107μg/kg) at day 21. Despite this evidence for some systemic uptake of neomycin following intramammary infusion, there is no evidence for toxicity, possibly because residues do not persist in the kidneys at toxic concentrations for long enough. It is concluded that intramammary infusions of neomycin reflect good practice in the use of veterinary drugs.

The sponsor's HPLC method for quantifying neomycin in milk is suitable for regulatory purposes. Moreover, there was good agreement between the results of the HPLC and microbiological methods, demonstrating that microbiological assays would be suitable for preliminary analyses of large numbers of milk samples in regulatory programs. Five qualitative tests were assessed for their suitability for screening commercial milk supplies for neomycin residues approaching the MRL. The Brilliant Black Reduction, Penzym and Valio T101 tests were negative within 84 hours of the last intramammary infusion whereas the Dutch Tube Diffusion and Delvotest® SP tests were positive at 120 hours after the last treatment. False-positive results with mastitic milk were observed with the Penzym, Valio T101, and Dutch Tube Diffusion tests. It appeared from the study that the Brilliant Black Reduction test would be suitable for screening commercial milk supplies for neomycin exceeding the MRL.

The study into the depletion of neomycin residues from kidneys was conducted in non-ruminating bull calves weighing about 35 kg, given neomycin sulphate orally at a dose equivalent to 15 mg/kg bw as neomycin base for 14 consecutive days. Although this study was initially designed for the determination of residues in kidneys only, liver samples were also analysed for neomycin residues albeit 16 months later. Stability data for liver residues during storage were not provided. No conclusions could be drawn from the study on account of the paucity and quality of the data generated.

Maximum Residue Limits

The Committee considered the following factors in recommending MRLs

- An ADI of 0 – 60 μg/kg bodyweight based on a toxicological endpoint, which results in a maximum daily intake of 3,600 μg for a 60 kg person.
- Neomycin undergoes negligible metabolism following parenteral administration to animals and the parent drug represents the total of the residues present.
- Neomycin is the marker residue for tissues, milk and eggs.
- A validated HPLC method with a LOQ of 0.1 μg/mL for neomycin in cows' milk is available that could be used routinely in many laboratories.
- Concentrations of neomycin up to 2 mg/L had no effect on bacterial starter cultures used in the production of fermented milk products.
- Data on residues in milk supported an MRL for cows' milk of 1,500 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 52nd Meeting to accommodate use of parenteral formulations are therefore unnecessary.

The Committee, having considered the database submitted since its 43rd meeting, decided to revert to the MRLs for cattle kidney and liver that it had recommended at its 47th 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, 1,500 μg/kg. The MRLs of 500 μg/kg for cattle muscle and fat were maintained.

Based on the 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 is 3,025 μg (Table 8). This accounts for 84 % of the ADI of 3,600 μg for a person of 60 kg bodyweight.

Table 8. Theoretical maximum daily intake (TMDI) of neomycin residues

Tissue	Food Basket (kg)	MRL (μg/kg)	Intake (μg)
Muscle	0.300	500	150
Liver	0.100	500	50
Kidney	0.050	10,000	500
Fat	0.050	500	25
Milk	1.500	1,500	2,250
Eggs	0.100	500	50
Total			3,025

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ANNEX 1

SUMMARY OF JECFA EVALUATIONS OF VETERINARY DRUG RESIDUES FROM THE 32ND MEETING TO THE PRESENT

This following table summarises the veterinary drug evaluations conducted by JECFA at the 32nd (1987), 34th (1989), 36th (1990), 38th (1991), 40th (1992), 42nd (1994), 43rd (1994), 45th (1995), 47th (1996), 48th (1997), 50th (1998), 52nd (1999), 54th (2000), 58th (2002), and 60th (2003) meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in food. **This table must be considered in context with the full reports of these meetings, which are published as WHO Technical Report Series.**

Some notes regarding the Table:

- The "ADI Status" column refers to the ADI and indicates whether an ADI was established, if a full ADI was given, or if the ADI is temporary (T).
- Where an MRL is temporary, it is so indicated by "T".
- Several compounds have been evaluated more than once. The data given are for the most recent evaluation, including the 60th meeting of the Committee.

A comprehensive listing of references to all JECFA evaluations and publications is available from the on-line edition of the *Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1956-2001)* which can be accessed from FAO and WHO websites for JECFA (www.fao.org/es/esn/jecfa/index_en.stm and www.who.int/pcs/jecfa/jecfa.htm).

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Abamectin	0-1 (1995 JMPR)	Full	47 (1996)	100 50	Liver, fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	0-50	Full	34 (1989)	100 5000	Muscle, fat, milk Liver, kidney	Cattle, sheep	MRLs analysed as 2-amino-benzimidazole and expressed as parent drug equivalents, see WHO TRS 788
Azaperone	0-6	Full	50 (1998)	60 100	Muscle, fat Liver, kidney	Pigs	Sum of azaperone and azaperol
Benzylpenicillin	30 μg/person/day	Full	36 (1990)	50 4	Muscle, liver, kidney Milk	All species	Parent drug
Bovine Somatotropins	Not specified	Full	50 (1998)	Not specified	Muscle, liver, kidney, fat, milk	Cattle	
Carazolol	0-0.1	Full	43 (1994)	5 25	Muscle, fat/skin Liver, kidney	Pigs	Parent drug. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI which is based on the acute pharmacological effect of carazolol
Carbadox	No ADI		60 (2003)	No MRL			Quinoxaline-2-carboxylic acid
Ceftiofur	0-50	Full	45 (1995) 48 (1997)	 1000 2000 6000 2000 100 μg/l	 Muscle Liver Kidney Fat Milk	 Cattle, pigs Cattle	Desfuroylceftiofur
Cefuroxime	0-30	T	58 (2002)	50 μg/kg	Milk	Cattle	Parent drug
Chloramphenicol	No ADI		42 (1994)	No MRL			
Chlorpromazine	No ADI		38 (1991)	No MRL			
Chlortetracycline, oxytetracycline, tetracycline	0-30 (Group ADI)	Full	58 (2002)	200 600 1200 400 100 μg/l 100 200	Muscle Liver Kidney Eggs Milk Muscle Muscle	Cattle, pigs, sheep, poultry Poultry Cattle, sheep Giant prawn Fish	Parent drugs, singly or in combination Oxytetracycline only

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Clenbuterol	0-0.004	Full	47 (1996)	0.2 0.6 0.05 μg/l	Muscle, fat Liver, kidney Milk	Cattle, horses Cattle	Parent drug
Closantel	0-30	Full	36 (1990) 40 (1992)	 1000 3000 1500 5000 2000	 Muscle, liver Kidney, fat Muscle, liver Kidney Fat	 Cattle Sheep	Parent drug
Cyfluthrin	0-20	Full	48 (1997)	20 200 40 μg/l	Muscle, liver, kidney Fat Milk	Cattle	Parent drug
Cyhalothrin	0-2	T	58 (2002)	20 T 400 T 30 μg/kg T	Muscle, liver, kidney Fat Milk	Cattle, pig, sheep Cattle	Parent drug
Cypermethrin	0-50	Full	58 (2002)	20 200	Muscle, liver, kidney Fat	Sheep	Parent drug
α-Cypermethrin	0-20	Full	58 (2002)	100 1000 100	Muscle, liver, kidney Fat Milk	Cattle, sheep Sheep	Parent drug
Danofloxacin	0-20	Full	48 (1997)	200 400 100 100 50 200 100	Muscle Liver, kidney Fat Muscle Liver Kidney Fat	Cattle, chickens Pigs	Parent drug For chickens fat/skin in normal proportion
Deltamethrin	0- 10 (JMPR 1982)	Full	60 (2003)	30 50 500 30 30	Muscle Liver, kidney Fat Milk Egg	Cattle, sheep, chicken, salmon Cattle, sheep, chicken Cattle, sheep, chicken Cattle Chicken	Parent drug

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Dexamethasone	0-0.015	Full	50 (1998)	No MRL			Temporary MRLs were not extended Regulatory method not available
Diclazuril	0-30	Full	50 (1998)	500 3000 2000 1000	Muscle Liver Kidney Fat	Sheep, rabbits, poultry	Parent drug Poultry skin/fat
Dicyclanil	0-7	Full	60 (2003)	150 125 200	Muscle Liver, kidney Fat	Sheep	Parent drug
Dihydrostrepto-mycin, streptomycin	0-50 (Group ADI)	Full	58 (2002)	600 1000 200	Muscle, liver, fat Kidney Milk	Cattle, pigs, sheep, chickens Cattle, sheep	Sum of dihydrostreptomycin and strepto-mycin
Demetridazole	No ADI		34 (1989)	No MRL			
Diminazene	0-100	Full	42 (1994)	500 12000 6000 150 μg/l	Muscle Liver Kidney Milk	Cattle	Parent drug
Doramectin	0-0.5	Full	58 (2002)	10 5 100 30 150	Muscle Muscle Liver Kidney Fat	Cattle Pigs Cattle, Pigs	Parent drug. The Committee noted the high concentration of residues at the injection site over a 35-day period after subcutaneous or intramuscular administration of the drug at the recommended dose.
Enrofloxacin	0-2	Full	48 (1997)	No MRL			
Eprinomectin	0-10	Full	50 (1998)	100 2000 300 250 20 μg./l	Muscle Liver Kidney Fat Milk	Cattle	Eprinomectin B_{1a}
Estradiol-17β	0-0.05	Full	52 (1999)	Not specified	Muscle, liver, kidney, fat	Cattle	
Febantel, fenbendazole, oxfendazole	0-7 (Group ADI)	Full	50 (1998)	100 500 100 μg/L	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs, horses, goats Cattle, sheep	Sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as ox-fendazole sulfone equivalents

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Fenbendazole (see febantel)							
Fluazuron	0-40	Full	48 (1997)	200 500 7000	Muscle Liver, kidney Fat	Cattle	Parent drug
Flubendazole	0-12	Full	40 (1992)	10 200 500 400	Muscle, liver Muscle Liver Eggs	Pigs Poultry	Parent drug
Flumequine	No ADI		60 (2003)				Parent drug
Furazolidone	No ADI		40 (1992)	No MRL			
Gentamicin	0-20	Full	50 (1998)	100T 2000 5000 200 μg/l	Muscle, fat Liver Kidney Milk	Cattle, pigs Cattle	Parent drug
Imidocarb	0-10	Full	50 (1998)	300 T 2000 T 1500 T 50 T 50 μg/L T	Muscle Liver Kidney Fat Milk	Cattle	Parent drug
Ipronidazole	No ADI		34 (1989)	No MRL			
Isometamidium	0-100	Full	40 (1992)	100 500 1000	Muscle, fat, milk Liver Kidney	Cattle	Parent drug
Ivermectin	0-1	Full	58(2002)	100 40 15 20 10	Liver Fat Liver Fat Milk	Cattle Pigs, sheep Cattle	Ivermectin B_{1a}
Levamisole	0-6	Full	42 (1994)	10 100	Muscle. Kidney, fat Liver	Cattle, sheep, pigs, poultry	Parent drug

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Lincomycin	0-30	Full	58 (2002)	200 500 1500 500 100 150	Muscle Liver Kidney " Fat Milk	Chickens, pigs " Pigs Chicken Chickens, pigs Cattle	Parent drug A separate MRL of 300 μg/kg for skin with adhering fat in pigs was recommended in order to reflect the high concentrations found in the skin of pigs. For consistency, an MRL of 300 μg/kg for skin with adhering fat in chickens was also recommended.
Melengestrol acetate	0-0.03	Full	58 (2002)	2 5	Liver Fat	Cattle	Parent drug
Metronidazole	No ADI		34 (1989)	No MRL			
Moxidectin	0-2	Full	50 (1998)	100 50 500 50 20 20 100 50 500	Liver Kidney Fat Muscle Muscle Muscle Liver Kidney Fat	Cattle, sheep Sheep Cattle Deer	Parent drug. The Committee noted the very high concentration and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.
Neomycin	0-60	Full	60 (2003)	500 500 10000 500 1500	Muscle, fat Liver Kidney Eggs Milk	Cattle, chicken, duck, goat, pig, sheep, turkey Cattle, chicken, duck, goat, pig, sheep, turkey Cattle, chicken, duck, goat, pig, sheep, turkey Chicken Cattle	Parent drug
Nicarbazin	0-400	Full	50 (1998)	200	Muscle, liver, kidney, fat/skin	Chicken (broilers)	

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Nitrofurazone	No ADI		40 (1992)	No MRL			
Olaquindox	Limited acceptance	T	42 (1994)	No MRL (see remarks)	Muscle	Pigs	MQCA[1]. The Committee recommended no MRLs but noted that 4 μg/kg of MQCA (T) is consistent with Good Veterinary Practice
Oxfendazole (see febantel)							
Oxolinic acid	No ADI		43 (1994)	No MRL			
Oxytetracycline (see chlortetracycline)							
Phoxim	0-4	Full	58 (2002)	50 400 50 T 400 T 10	Muscle, liver, kidney Fat Muscle, liver, kidney Fat Milk	Goats, pigs, sheep " Cattle " "	Parent drug
Porcine somatotropins	Not specified		52 (1999)	Not specified	Muscle, liver, kidney, fat	Pigs	
Procaine benzylpenicillin	Less than 30 μg of penicillin per person per day	Full	50 (1998)	50 4 μg/kg	Muscle, liver, kidney Milk	Cattle, pigs, chickens Cattle	Benzylpenicillin
Progesterone	0-30	Full	52 (1999)	Not specified	Muscle, liver, kidney, fat	Cattle	
Propionyl-promazine	No ADI		38 (1991)	No MRL			
Ractopamine	No ADI		40 (1992)	No MRL			
Ronidazole	Withdrawn		42 (1994)	No MRL			
Sarafloxacin	0-0.3	Full	50 (1998)	10 80 20	Muscle Liver, kidney Fat	Chicken, turkey Chicken, turkey Chicken, turkey	Parent drug
Spectinomycin	0-40	Full	50 (1998)	500 2000 5000 2000 200 μg/kg	Muscle Liver, fat Kidney Eggs Milk	Cattle, pig, sheep, chicken Chicken Cattle	Parent drug

[1] MQCA: 3-methylquinoxaline-2-carboxylic acid

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Spiramycin	0-50	Full	48 (1997)	200 600 300 800 300 200 μg/kg	Muscle Liver Kidney Kidney Fat Milk	Cattle, chicken, pig Cattle, chicken, pig Cattle, pig Chicken Cattle, chicken, pig Cattle	For cattle and chickens MRLs are expressed as the sum of spiramycin and neospiramycin For pigs MRLs expressed as spiramycin equivalents (antimicrobially active residues)
Streptomycin (see dihydrostrepto-mycin)							
Sulfadimidine	0-50	Full	42 (1994)	100 25 μg/kg	Muscle, liver, kidney, fat Milk	Cattle, sheep, pig, poultry Cattle	Parent drug
Sulphthiazole	No ADI		34 (1989)	No MRL			
Testosterone	0-2	Full	52 (1952)	Not specified	Muscle, liver, kidney, fat	Cattle	
Tetracycline (see Chlortetracycline)							
Thiamphenicol	0-5	Full	58 (2002)				The temporary MRLs in muscle, liver, kidney and fat of pigs and muscle of fish were not extended because the information requested at the fifty-second meeting (WHO TRS 893, 2000) was not provided.
Tiabendazole	0-100	Full	58 (2002)	100 100 μg/kg	Muscle, liver, kidney, fat Milk	Cattle, pig, goat, sheep Cattle, goat	Sum of thiabendazole and 5-hydroxythiabendazole
Tilmicosin	0-40	Full	47 (1996)	100 1000 1500 300 1000 50 μg/kg T	Muscle, fat Liver Liver Kidney Kidney Milk	Cattle, pig, sheep Cattle, sheep Pig Cattle, sheep Pig‘ Sheep	Parent drug
Trenbolone acetate	0-0.02	Full	34 (1989)	2 10	Muscle Liver	Cattle	β-Trenbolone for muscle α-trenbolone for liver

Substance	ADI (μg/kg bw)	ADI Status	JECFA	MRL (μg/kg)	Tissue	Species	Marker residue and other remarks
Trichlorfon (Metrifonate)	0-2	Full	60 (2003)	50 μg/kg 50	Milk Muscle, liver, kidney, fat	Cattle Cattle	Parent drug Guidance MRLs (No residues detected in depletion studies. No residues should be present in tissues when used with good veterinary practice. Limit of quantification used as guideline MRL)
Triclabendazole	0-3	Full	40 (1992)	200 300 100 100	Muscle Liver, kidney Fat Muscle, liver kidney, fat	Cattle Sheep	5-Chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one
Tylosin	No ADI		38 (1991)	No MRL			
Xylazine	No ADI		47 (1996)	No MRL			
Zeranol	0-0.5	Full	32 (1987)	2 10	Muscle Liver	Cattle	Parent drug

ANNEX 2

SUMMARY OF RECOMMENDATIONS FROM THE 60th JECFA ON COMPOUNDS ON THE AGENDA AND FURTHER INFORMATION REQUIRED

Antimicrobial agents

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.

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)[a]

Species	Liver (µg/kg)	Kidney (µg/kg)	Milk (µg/kg)
Cattle	500	10 000	1500

[a]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.

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)

Species	Fat (µg/kg)	Kidney (µg/kg)	Liver (µg/kg)	Milk (µg/kg)	Muscle (µg/kg)
Cattle	50	2000	1500	50	300

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 Papers 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 400 µg, equivalent to 67% 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.

Dicyclanil

Acceptable daily intake: 0-0.007 mg/kg bw (established at the fifty-fourth meeting of the Committee (WHO TRS 900, 2001))

Residue definition: Dicyclanil

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Sheep	150	125	125	200

Trichlorfon (metrifonate)

Acceptable daily intake: The Committee amended the ADI for trichlorfon from 0-20 µg/kg bw 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).

ANNEX 3

GENERAL CONSIDERATION ITEMS

Withdrawal of ADIs and MRLs for flumequine and carbadox

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its sixtieth meeting decided not to continue support for the MRLs for flumequine and carbadox. A review of new information on the toxicology and residue chemistry of these two compounds led the Committee to conclude that it could not support the ADIs and MRLs for carbadox and flumequine that it had recommended at previous meetings.

Notwithstanding this decision, consumers should have every confidence that there is no evidence that any harmful effects have been caused by residues of either of these two compounds that may have been present in food resulting from approved uses in animals. The concentrations of residues were many times lower than those shown to have any effects in experimental systems or animals.

Considerations on marker residues

The Committee at its fortieth meeting 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 concentration 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 principle on which the definition used by the Committee is based is that in virtually all instances a 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.