Residues of some veterinary drugs in animals and foods

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JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (62nd Meeting)

Rome, 4–12 February 2003

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ABBREVIATIONS

3-PBA	3-phenoxbenzoic acid
4'-OH-3-PBA	4-hydroxy-3-phenoxybenzoic acid
ADI	Acceptable Daily Intake
AOAC	AOAC International (Association of Analytical Communities)
APCI	Atmospheric pressure chemical ionisation
AQC	Analytical quality control
AR	Human androgen receptor
AUC	Area under the curve
AUC	area under the curve
bw	Body weight
CAS	Chemical Abstracts Service
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CCRVDF	
ClB	Clearance blood
Cmax	Maximum concentration
CR	Cearance renal
CV	Coefficient of variation
Cyp	Cypermethrin
dpm	decays per minute
ECD	Electron Capture Detector
EDTA	ethylenediaminetetraacetic acid
ELISA	Enzyme linked immuno sorbent assay
EMEA	European Agency for the Evaluation of Medicinal Products
ERα	Human oestrogen receptor α-subtype
FDA	US Food and Drug Admistration
GC	Gas chromatography
GLP	Good Laboratory Practice
GR	Human glucorticoid receptor
HCC	High cis cypermethrin
HPLC	High pressure liquid chromatography
IEC	Ion exchange chromatography
IMM	Intramammary
IR	Infrared
IV	Intravenous
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
MGA	Melengestrol acetate
MIC	Minimal Inhibitory Concentration
MRL	Maximum Residue Limit
MS	Mass spectrometry
N	Negative
NA	Not applicable or Not assayed
NADA	New animal drug application
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
ND	Not detected
NI	Not investigated
NICI	negative ion chemical ionization
NMR	nuclear magnetic resonance
NQ	Not quantifiable
OECD	Organisation for Economic Co-operation and Development
P	Positive
PES	Post-extracted solids
PR	Human progesterone receptor B-subtype
QC	Quality control
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
TSP	Thernospray
USP	United States Pharmacopoeia
Vd	Volume of distribution

INTRODUCTION

The monographs on the residues of, or statements on, the veterinary drugs contained in this volume were prepared by the 62nd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Rome, 4–12 February 2004. 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], 58th[20] and 60th[21]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, DC 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, fifteen meetings of JECFA were dedicated exclusively to evaluation of veterinary drugs.

62nd Meeting of JECFA

The present volume contains monographs of the residue data on eight of the eleven 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 recommendations on compounds on the agenda and further information required is included in Annex 2, General Consideration Items are published in Annex 3.

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.

Summary of JECFA evaluations of veterinary drug residues from the 32nd meeting to the present 62nd meeting is found in Annex 1.

Substances without ADI/MRL

Following a request from the 14th session of the Codex Committee on Residues of Veterinary Drugs in Foods (ALINORM 03/31A para 93) an additional Annex 4 was added that lists all substances for which the Committee could not allocate an ADI or propose MRL.

On-line edition of ***Residues of some veterinary drugs in animals and foods (FAO Food and Nutrition paper 41)***

The monographs and statements that have been published in the FAO Food and Nutrition Paper 41 (sixteen volumes since 1988) are available 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 searching 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.

Melengestrol acetate

During the editing of the monograph for melengestrol acetate (MGA) some inconsistencies in the approach to derive the activity weighing factors for MGA-related residues where detected which could be corrected partially. To address all of them requires a revision by the next meeting of JECFA that will assess residues of veterinary drugs.

Contact and 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:

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REFERENCES

1. Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation: Some antibiotics (Twelfth Report of the Joint FAO/WHO Expert Committee on Food Additives), FAO Nutrition Meetings Report Series No. 45, 1969; WHO Technical Report Series No. 430, 1969.
2. Evaluation of Certain Food Additives and Contaminants (Twenty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 683; 1982.
3. Evaluation of Certain Food Additives and Contaminants (Twenty-Seventh Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 696; 1983.
4. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 763; 1988.
5. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Fourth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 788; 1989.
6. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 799; 1990.
7. Evaluation of Certain Veterinary Drug Residues in Foods. (Thirty-Eighth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 815; 1991.
8. Evaluation of Certain Veterinary Drug Residues in Foods (Fortieth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 832; 1993.
9. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 851; 1995.
10. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-third Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 855; 1995.
11. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-fifth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 864; 1996.
12. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-seventh Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 876; 1998.
13. Evaluation of Certain Veterinary Drug Residues in Foods (Forty-eighth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 879; 1998.
14. Evaluation of Certain Veterinary Drug Residues in Foods (Fiftieth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 888; 1999.
15. Evaluation of Certain Veterinary Drug Residues in Foods (Fifty-second Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 893, 2000.
16. Residues of Veterinary Drugs in Foods, Report of a Joint FAO/WHO Consultation, Rome, 29 October - 5 November 1984. FAO Food and Nutrition Paper No. 32, 1985.
17. Report of the First Session of the Codex Committee on Residues of Veterinary Drugs in Foods. Washington, D.C., 27-31 October 1986.
18. Report: JECFA/JMPR Informal Harmonization Meeting, 1-2 February, 1999, Rome, Italy, 1999.
19. Evaluation of Certain Veterinary Drug Residues in Foods (Fifty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 900, 2001.
20. Evaluation of certain veterinary drug residues in food (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 900, 2001.
21. Evaluation of certain veterinary drug residues in food (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.

CEFUROXIME SODIUM

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ADDENDUM

to the monograph prepared by the 58th meeting of the Committee and published in

FAO Food and Nutrition Paper 41/14

INTRODUCTION

Cefuroxime is a cephalosporin with activity against a wide range of Gram-positive and Gram-negative bacteria. It is used in veterinary medicine by intramammary administration (as sodium salt) for the treatment of mastitis. In human medicine it is also used as the 1-(acetoxy)ethyl ester of the drug (cefuroxime axetil) for the treatment of different bacterial infections.

Cefuroxime sodium is available for veterinary use as Spectrazol™ Dry Cow (12,5% cefuroxime sodium) and Spectrazol™ Wet Cow (9,22% cefuroxime sodium). Spectrazol™ Dry Cow is a long-acting formulation intended for treatment of subclinical mastitis in dry cows and for prevention of new infections during the dry period. It is used as a single dose syringe (375 mg of cefuroxime) for intramammary administration, for a total dose of 1.5 g per animal. Spectrazol™ Wet Cow is used in the treatment of clinical mastitis and is supplied as a syringe (250 mg cefuroxime sodium) for intramammary administration, for a total dose of 1 g per animal, per day.

For the first time cefuroxime was evaluated by the 58th meeting of JECFA in 2002. The Committee established a temporary microbiological ADI of 0-30 µg per kg of body weight and recommended a temporary MRL of 50 µg/kg for cefuroxime parent compound in milk from cattle. The available data were not sufficient to permit the establishment of MRLs for other edible tissues (JECFA, 2002).

The Committee requested the following information to be provided by 2004:

1. Data to clarify whether the residues other than parent compound found in bovine milk are due primarily to metabolism or to non-metabolic decomposition of parent cefuroxime in milk;
2. Identification of the non-parent residues found in bovine milk, including an assessment of their potential toxicity.

None of the requested information was provided to the 62nd meeting of the Committee. The Sponsor argued that the information requested by the 58th JECFA could be provided on the basis of additional interpretations of previously submitted data and without the conduct of further studies. The Sponsor re-submitted the key reports included in the previous dossier. In addition, scientific articles published in the open literature on: (1) pharmacokinetics of cefuroxime in humans, (2) stability of cefuroxime in liquid environment, and (3) HPLC analytical methods for cefuroxime determination in blood plasma, were made available by the Sponsor, in response to a request of the drafting expert of the Committee.

Since no new information had been provided to the Committee and the additional interpretations offered by the Sponsor did not adequately address the problems identified by the 58th JECFA, further questions for clarification were raised by the experts of the Committee and written responses were received from the Sponsor. It was re-emphasized by the Committee that in the report of the pivotal study of Ferguson and Batten (1996) there were still serious unexplained discrepancies in the sets of analytical data obtained with direct samples of individual milkings and with pools of individual milkings, respectively. Some of these discrepancies occurred although the same validated method had been used (HPLC-MS). In other cases different methods were used which should produce essentially equivalent results (HPLC-radioanalysis vs. HPLC-MS). The discrepancies could not be resolved on the basis of the submissions to the 58th and 62nd JECFA. They could be resolved if one would assume that a major part of the residues representing parent drug had gradually decomposed during storage and treatment of the samples. The Sponsor accepted that there was an unexplained discrepancy in the results of the analysis of milk obtained at the critical fifth milking after the last infusion. Furthermore the responses of the Sponsor revealed that certain critical samples underwent several freezing-thawing cycles during storage and further processing. In fact, on the basis of the responses of the Sponsor the Committee could no longer exclude that degradation of residues occurred post-sampling, possibly during thawing/freezing cycles to an extent that the results obtained and reported for the residue composition of stored milk are not representative of the residue composition of fresh milk taken directly from the cow. The additional information contained in the responses of the Sponsor partly invalidate the pivotal study of Ferguson and Batten. Only the results of measurements of radioactivity in samples of milk appear to be fully reliable in this study. This conclusion should be kept in mind when reading the following part of this document.

RESPONSE OF THE SPONSOR TO THE REQUEST FOR

INFORMATION BY THE 58TH MEETING OF JECFA

1. Clarification whether the residues other than parent compound found in milk are primarily due to metabolism or to non-metabolic decomposition of parent cefuroxime in milk environment

The response of the Sponsor was submitted in the form of an expert report. This report stated that the main objective of the study in which [^{14}C]-cefuroxime had been intramammary infused to lactating cattle (Ferguson and Batten, 1996), was to determine the concentrations of antibacterially active residues, including the parent compound and to establish relationships between the concentrations of parent compound, other antibacterially active residues, and total radioactive residues of cefuroxime. The need to characterize or identify components that were devoid of antibacterial activity was not perceived as being important, given the emphasis being placed on antibacterially active residues (Parker, 2003). However, the Sponsor could also not provide information on the microbiological activity of potential metabolites or breakdown products.

From the analysis of milk obtained at different time points after administration of ^{14}C-cefuroxime it was found that cefuroxime was transformed into other compounds. The parent compound was a significant component of the antibacterially active residues up to the 3rd milking after the last administration of cefuroxime, however marked differences between results obtained by the HPLC methods and a microbiological assay have not been explained by the Sponsor. Samples of the milk of all eight cows used in the experiment were taken at the 5th milking after the last cefuroxime infusion to prepare a pool. The concentrations of cefuroxime in six samples were above the limit of quantification of the analytical method (HPLC-MS), with a mean of 0.143 µg/g and a range from 0.011 to 0.328 µg/g. Contrary to expectations the concentration in the pool was below 0.01 µg/g (Ferguson and Batten, 1996) meaning that more than 90% of the parent drug had disappeared during the time period between the analysis of the individual samples and the analysis of the pool.

Liquid chromatographic analysis of milk samples taken up to the 5th milking indicated that – at the time of analysis - more than 80% of the radiolabelled residues were unidentified substances**.** When samples of subsequent milkings were analyzed, the parent compound was almost not detected, while more cefuroxime transformation products were present. According to the expert report identification of the radioactive components in milk other than unchanged parent compound was not performed and samples have been destroyed (Parker, 2003).

Concerning the possibility of non metabolic degradation of cefuroxime in milk environment, the submission claimed that the published scientific evidence is equivocal (Parker, 2003). Okumura *at al.* (1979) incubated cefuroxime with rat plasma or with homogenates of rat liver, kidney, spleen or lung in phosphate buffered saline at pH 7.2 and 37 ^{o}C for up to 90 minutes and recovered 90% or more of the parent compound. The Merck Index (1989) notes that aqueous solutions of sodium cefuroxime are stable at room temperature for 13 hours, and that less than 10% decomposition occurs in 48 hours at 25 ^{o}C. However, repeated measurement of cefuroxime in milk samples from the study of Ferguson and Batten indicated that concentrations decreased significantly over a storage period of two weeks. This finding was attributed to differences in batch recoveries of the HPLC-MS method by the authors of the study (Ferguson and Batten, 1996). However, according to the expert report, these decreases were probably too great to be fully accounted for in this way, and it is also possible that the cefuroxime was lost either during freezer storage or during freeze-thawing or due to bacterial metabolism in the non-sterile samples (Parker, 2003).

According to the expert report transformation of cefuroxime in the udder is too great to be accounted for solely, or even predominantly, by a non-enzymatic mechanism. Even if cefuroxime is unstable to some extent in the environment of the mammary gland, there are no reasons for supposing that it would not be similarly unstable in other biological matrices. It is unlikely that the fluids in the udder possess any qualities that result in more rapid non-enzymatic degradation of cefuroxime than would be seen, for example, in blood (Parker, 2003).

Results of pharmacokinetic studies in rats, dogs and humans show that cefuroxime is rapidly and almost completely (80-95% of the dose) excreted unchanged (> 95%) in urine during 24-72 hours (Foord, 1976; Gower and Dash, 1977; Nanbo *et al.*, 1979), while it is extensively metabolized and slowly eliminated with milk after being infused into the udders of lactating cows (Ferguson and Batten, 1996). According to the expert report, the differences are not due to the animal species, but to the route of administration, and it is probable that in rats, dogs and humans the drug is rapidly excreted before there is an opportunity for significant metabolism to take place. The nominal 12 hours between milkings in cows is a long time for a drug that is normally eliminated with a short half-life, and it is very likely that metabolism could occur that would not have taken place if the drug had been administered by a route that allowed rapid excretion (Parker, 2003). Furthermore, results of pharmacokinetic studies in cattle show that cefuroxime is rapidly eliminated after being administered by intravenous or intramuscular injection with a half-life ($t_{1/2\beta}$) of 1.15-1.47 hours (Silley and Rudd, 1986; Soback *et al.*, 1989; Chaudhary *et al.*, 2001). The routes by which cefuroxime is metabolized within mammary gland are not unique and are similar to metabolic pathways available for transformation of cefuroxime in rats, dogs and humans after its non-intramammary administration. Cefuroxime is not resistant to metabolism, but most of the dose escapes transformation because it is cleared from the body before metabolism can occur, however, it can not be ruled out that some of the metabolites in milk are unique to cattle dosed by intramammary infusion (Parker, 2003).

2. Identification of the non-parent residues found in milk, including an assessment of their potential toxicity

In humans and different animal species cefuroxime, when administered orally as the axetil derivative or parenterally as the sodium salt, is rapidly and almost completely excreted in urine during 24-72 hours. According to the Sponsor's expert report, cefuroxime metabolism occurs in all species, but the rate of this process is generally slower than the rate of excretion. However, if excretion is "blocked" by trapping the cefuroxime in the udder, than the results of metabolism become more evident. Whether these metabolites are exactly the same in different animal species and in humans, as those found in bovine milk after intramammary administration, cannot be known because there are no results from comparative metabolism studies. If some of the metabolites of cefuroxime are different, than this creates concerns about their toxicity (Parker, 2003).

Results of the study with radiolabelled cefuroxime in cattle suggest that the concentrations of parent compound in milk decrease faster than the total radioactive residues. The expert report assumes that a concentration of parent compound in milk equivalent to the temporary MRL of 50 μg/kg corresponds to a "total residue" concentration of approximately 3 mg equivalents/kg (Parker, 2003). Assuming human consumption of 1.5 kg of milk per day, this total milk residue equates to a daily intake of 4.5 mg equivalents, which is less than 1.9% of the temporary toxicological ADI of 240 mg per 60 kg person established by the 58th meeting of the JECFA. Even if the metabolites of cefuroxime were 50 times more toxic than the mixture of parent compound and metabolites to which laboratory animals were exposed in toxicity studies, they would not pose a risk to human health. However, there are no reasons to believe that the non-parent residues of cefuroxime in milk would be more toxic than unchanged cefuroxime (Parker, 2003).

According to the expert report tissue residues of cefuroxime also need to be combined with the ADI. Due to limited absorption from the udder (< 20% of the dose), tissue residues are low, however, actual data on concentrations of "total residue" are not available for the period shortly after infusion of cefuroxime. Considering the data from the radiolabelled study (Ferguson and Batten, 1996), 24 hours after first infusion of cefuroxime about 4% of the dose had been excreted in urine and faeces, and at least 78% of the dose had been excreted either in milk or was located within mammary gland. Thus, less than 18% (540 mg) of the total dose of cefuroxime may have been absorbed from the udder into the blood, but not yet excreted. Assuming that all of absorbed dose was concentrated in the kidneys and that the two kidneys of a mature cow weight together 1.6 kg, than the concentration of total cefuroxime-derived residue in kidneys would have been estimated as 338 mg equivalents/kg. Assuming a daily intake of 50 g of kidney per day, the intake of residues by a 60 kg consumer would be 17 mg equivalents, which amounts to only 7% of the temporary toxicological ADI established by the 58th meeting of the JECFA. This assumption is clearly an exaggeration and takes no account of residues located in non-edible tissues, organs or body fluids, so in reality, it is unlikely that the edible tissues residues use up more than 1% of the temporary toxicological ADI. It is also unlikely that a cow will be slaughtered so soon after treatment, except in emergency. Furthermore, results of the study with radio-labeled cefuroxime in cows show that tissue residues are very low 7 days after treatment with cefuroxime (Table 1). Assuming daily consumption of 100 g of liver, 50 g of kidney, 300 g of muscle and 50 g of fat, these total residues account for only 0.007% of the temporary toxicological ADI or only 1% of the temporary microbiological ADI. Based on available information, it could be concluded that 50 μg/kg of cefuroxime would be a suitable concentration for a permanent MRL for bovine milk and it was not necessary to set MRL values for edible tissues in cattle (Parker, 2003).

Table 1. Mean total cefuroxime-derived residues in tissues of eight cows 7 days after the last of three successive intramammary doses of 1,000 mg [14C]-cefuroxime (Ferguson and Batten, 1996).

Tissue	Mean residue ± SD (mg equivalents/kg)
Liver	0.035 ± 0.011
Kidneys	0.101 ± 0.046
Muscles	< 0.020
Fat	< 0.060

Assessment of the Sponsors response to Question 1

Results of studies in which [^{14}C]-cefuroxime was intramammary infused in cows show that antibacterially active residues (including the parent compound) are eliminated with milk, however, concentrations below the temporary MRL for the parent compound of 50 μg/kg are not reached before the 5th milking. Moreover, based on HPLC analysis, more than 80% of the radiolabelled residues present in milk samples taken before the 5th milking were unidentified metabolites or degradation products of cefuroxime and in subsequent milkings amounts of cefuroxime transformation products were even higher (Ferguson and Batten, 1996).

Whether these metabolites or non-metabolic degradation products are exactly the same in different animal species and in humans cannot be known, because there are no results from comparative studies. If some of them are different, than this creates concerns about their toxicity.

The main arguments of the Sponsor concerning non-metabolic decomposition of cefuroxime in milky/liquid environment are the following. There is a deficit in specific experimental evidence for such a theory. Furthermore, existing results (Okumura *et al.*, 1979; The Merck Index, 1989; Ferguson and Batten, 1996) regarding cefuroxime stability are equivocal, and even if

cefuroxime is unstable to some extent in the milky environment of the udder there are no reasons for supposing that it would not be similarly unstable in other biological matrices (Parker, 2003).

Unfortunately, the Sponsor did not perform tests of the stability of cefuroxime in bovine milk. However, results of tests of the stability of ceftiofur (a third-generation cephalosporin) in milk and blood plasma indicate marked differences in long-term stability in these biological matrices (Erskine *et al.*, 1995). Ceftiofur lost 86.8% of bioactivity within 30 minutes following the addition to serum at 20 ^{o}C. Ceftiofur in serum stored at -20 ^{o}C continued to lose bioactivity, so that by 3 weeks of storage, only 1.2% of the original activity was detected. However, ceftiofur activity in milk, immediately after mixing and after 3 weeks of storage at -20 ^{o}C, did not differ from activity before storage (Erskine *et al.*, 1995).

In the study of Okumura *et al.* (1979) only about 90% of the drug was recovered after 90 minutes of incubation at 37 °C. In the study of Ferguson and Batten (1996) HPLC-MS measurements of cefuroxime in milk samples taken at the 5th milking following the last infusion of the drug were repeated within 14 days**.** The results of the second analysis were significantly lower in all samples ranging clearly above the LOQ of the method upon first analysis. The Sponsor confirmed these discrepancies (Parker 2004), but gave no satisfactory explanation for these findings which could be indicative for cefuroxime decomposition during storage.

Time dependent instability of cefuroxime at 4 ^{o}C was shown in long-term studies in 5% dextrose. After 11, 21 and 30 days of incubation, cefuroxime recoveries were 93.0, 89.6 and 78.3%, respectively (Galanti *et al.*, 1996). Schlesser *et al.* (2001) defined the time of cefuroxime stability in 5% dextrose solution as the time during which the lower confidence limits of the residual value remained superior to 90% of the initial concentration by regression analysis. The stability of cefuroxime solution was estimated at 13 days when stored at 4 ^{o}C (Schlesser *et al.*, 2001). In another study in 5% dextrose or in 0.9% NaCl injectable solution cefuroxime sodium was stable (96% recovery) at 5 ^{o}C during 30 days, however, at 25 ^{o}C recoveries amounted to only < 94, < 87 and < 75% after 1, 2 or 4 days, respectively. Stability test performed at -10 ^{o}C showed almost complete (> 99%) stability of cefuroxime (Das Gupta and Stewart, 1986). Similar results on stability of cefuroxime in aqueous solutions were obtained by Wang and Notari (1994). Maximum stability was observed in the pH range from 4 to 7, where the time during which cefuroxime concentration exceeded 90% of its initial concentration at 25 ^{o}C was 1.2 days. The predicted shelf-life for aqueous cefuroxime solutions in the pH range 4-7 as a function of temperature shows values of < 1 (30 ^{o}C), 2.5 (20 ^{o}C), 12.5 (10 ^{o}C) and 45 days (2.5 ^{o}C). In the same study descarbamoyl cefuroxime was identified as a degradation product of cefuroxime hydrolysis, which still possesses a ß-lactam ring (Wang and Notari, 1994). Barnes (1990) reported that at 25 ^{o}C in solutions for intravenous administration cefuroxime degraded by first-order kinetics with a rate constant of 7.04 x 10^{-2}/day and a t_{90} (time to reach 90% of the original concentration) of 36 hours. At 4 ^{o}C the rate constant was 5.23 x 10^{-3}/day with a t_{90} of 20 days. However, over the time-scale studied, the kinetics approximated to zero-order at this temperature. Jorgensen *et al.* (1988) provided stability data for cefuroxime in different media. Several examples are given in Table 2.

Oldham and Andrews (1996) described the preparation of a simple, unpreserved aqueous solution of cefuroxime sodium (equivalent to 5% cefuroxime) from Zinacef® injection and sterile water for injections. The eye drops were chemically stable for 24 hours at room temperature and for 21 days at 2 ^{o}C during simulated patient use. The authors concluded that solutions may be frozen at -30 ^{o}C for up to 12 months with negligible loss of antibacterial potency, and on thawing at room temperature the stability is maintained for 21 days at 2 ^{o}C or for 14 days at 8 ^{o}C.

From stability studies of 14 different cephalosporins (including cefuroxime) it was seen that within 4 hours after dissolving in water at room temperature (25 ^{o}C) their degradation (hydrolysis) is generally not higher than 20%. After 260 hours however, only < 2% of the original amount of the drug e.g. cefuroxime, was still present. Moreover, formation of four unknown hydrolysis products of cefuroxime (named as D_1-D_4) has also been observed. The D_1 degradation product showed almost total instability and disappeared together with the parent compound after 150 hours of incubation, while D_2, D_3 and D_4 degradation products started to form about 50 hours after dissolution in water (Gáspár *et al.*, 2002).

Table 2. Stability of cefuroxime in human body fluids and bacteriological media at three temperatures (Jorgensen et al., 1988).

Diluent	% of starting concentration at*:		
	-20 °C for 7 days	35 °C for 8 hours	35 °C for 18 hours
Pooled human serum**	-	84.0	-
Pooled human urine**	-	95.0	-
Distilled water	95.6	79.3	-
Mueller-Hinton brothsupplemented withCa and Mg cations	94.7	86.6	79.7
Trypticase soy broth	94.0	82.8	81.0
Thioglycolate 135 °C medium	91.0	87.0	68.2

* - Mean of duplicate determinations of two concentrations (20 and 80 µg/ml) of drug

** - pH of pools adjusted to 7.4

Coomber *et al.* (1982) developed an HPLC assay for cefuroxime. In operating instructions they suggest to "store the standard and sample solutions in a refrigerator until they are required for injection; under these conditions cefuroxime sodium decomposes at a rate of approximately 0.05% per hour". Lecaillon *et al.* (1982) reported the maximum storage time, which produced less than 5% degradation, as 14 days at 5 ^{o}C for standard solutions and > 5 hours at room temperature in the autosampler for treated plasma and urine samples.

All these results indicate temperature and time dependent instability of cefuroxime sodium in aqueous solutions (including biological matrices) with rapid degradation rates in temperatures above 30 ^{o}C.

Results of pharmacokinetic studies in rats, dogs and humans show that cefuroxime is rapidly and almost completely excreted in urine in unchanged form during 24-72 hours (Foord, 1976; Gower and Dash, 1977; Nanbo *et al.*, 1979), while it is extensively metabolised and slowly eliminated with milk after being infused into the udders of lactating cows (Ferguson and Batten, 1996). In the opinion of the Sponsor the difference is predominantly a result of the route of administration and it is probable that laboratory animals and humans excrete cefuroxime so rapidly, because there is no opportunity (sufficient persistence time) for significant metabolism to take place, and most of the dose escapes transformation (Parker, 2003).

No evidence to support this hypothesis was presented. However, studies of the pharmacokinetics of cefuroxime in human patients with severe renal insufficiency could probably clarify whether metabolism can occur if the rate of excretion is reduced.

Results of pharmacokinetic studies in healthy humans (in most cases creatinine clearance > 100 ml/min./1.73 m^2) or in patients with severe renal impairment (creatinine clearance < 20 ml/min./1.73 m^2) are presented in Table 3. Analysis of these results generally indicates a good comparability of the results obtained by different authors. In healthy humans cefuroxime is quickly eliminated from the blood plasma with a half-life of about 1.4 hour and almost all (93-100%) of parenterally administered dose is excreted in urine in unchanged form during 24 hours. Furthermore, results obtained by HPLC assay indicate that cefuroxime has almost total metabolic stability in the body and the only metabolite observed in human urine (1.3% of the dose during 8 hours) is Δ2-cefuroxime, a hydrolysis product of the cefuroxime axetil (Ishibiki *et al.*, 1990).

In patients with severe renal insufficiency however, elimination from the blood plasma and urinary excretion of cefuroxime are markedly reduced. Reported values for cefuroxime half-life in renal impairment are about 10-times higher (in individuals with tubular necrosis even up to 28.3 hours) than in normal patients (van Dalen, *et al.*, 1983), what is a result of almost proportional decrease of the total body clearance (Table 3). Proportional 10-times increase of the AUC values for unchanged cefuroxime in blood plasma has also been observed in renal failure, and 24-hour urinary excretion was reduced even below 20% of the administered dose (Table 3). Moreover, microbiological disc diffusion assay and HPLC assay for cefuroxime yielded statistically identical results for blood plasma, as well as for urine (Bundtzen *et al.*, 1981; Massias *et al.*, 1998).

It is interesting, however, that in patients with end-stage of renal failure the half-life of elimination is not infinitely high and never exceed 30 hours (Vree and Hekster, 1990). The elimination must therefore proceed by mechanism other than renal excretion (van Dalen *et al.*, 1979). The mechanism by which this non-renal elimination takes place is still unknown. Analysis of linear correlations between the renal and total body clearances of cefuroxime and creatinine clearance shows that in patients with severe renal failure extrarenal clearance remained almost constant and it amounted only 8.24 ml/min. (Walstad *et al.*, 1983).

All the above results on pharmacokinetics of cefuroxime in humans indicate that this compound has almost total metabolic stability in healthy humans as well as in patients with severe or even end-stage renal failure. It cannot be excluded however, that during long persistence in the body cefuroxime undergoes some biotransformation.

Therefore, the Sponsor statements that (1) "it is possible that in humans the drug is rapidly excreted before there is an opportunity for significant metabolism to take place" and (2) "most of the dose escapes transformation because it is cleared from the body before metabolism can occur" appear unjustified.

It is very likely that the difference in cefuroxime metabolism between e.g. humans and cattle are related to a route of administration and specific transformation processes (bacteria dependent metabolism?) within the udder during lactation. However, this cannot be explained based on examples of cefuroxime pharmacokinetics after non-mammary administration (even in severe renal failure), simply because the drug is a strong acid and do not penetrate easily from the blood into acidic environment of milk. Moreover, it has small volume of distribution (V_d) – below 0.3 l/kg b.w. (Bundtzen *et al.*, 1981; Chaudhary *et al.*, 2001). Results from human studies also show that cefuroxime concentrations in milk ranged from 0.09 to 0.59 μg/ml at 30 to 90 minutes after oral administration of 500 mg of cefuroxime axetil (Nakamura *et al.*, 1987).

This means that they were about 10-times lower than corresponding concentrations observed in blood plasma (Ishibiki *et al.*, 1990; Konishi *et al.*, 1993; Nix *et al.*, 1997). Unfortunately, Nakamura *et al.* (1987) used bioassay technique for cefuroxime determination in milk samples which makes impossible assessment in scale of potential metabolites separation.

Table 3. Pharmacokinetic parameters of cefuroxime in healthy humans or in patients with renal insufficiency.

Health status: H: Healthy RI: Renal insufficiency **Cl_{CR}:** Creatinine clearance **Dose and route**	**Cmax** (µg/ml)	**Tmax** (min.)	**T1/2** (h)	**Vd** (l/1.73 m2)	**ClB** (ml/min/ 1.73 m2)	**ClR** (ml/min/1.73 m2)	**AUC** (µg/ml/h)	**24 h urinary excretion** (% of dose)	**Reference**
H 500 mg i.v.	C0 = 82.7	T0 = 0	1.1	11.09		136	50.4	95.1	Foord, 1976
H 1000 mg i.v.	C0 = 181.4	T0 = 0	1.1	12.83		169.6	90.8	99.1	
H 500 mg i.m.	26.9	29	1.2	13.45		128.9	59.2	96.5	
H 750 mg i.m.	34.9	31	1.4	15.81		137.9	88.6	101.9	
H 1000 mg i.m.	40.4	45	1.1	15.05		146.3	101.3	103.4	
H 500 mg i.v.	42.0	15	1.1	11.7	148		53.1	93.1	Gower and Dash, 1977
H 750 mg i.v.	52.6	15	1.1	12.5	152		74.0	94.8	
H Cl_{CR} >115 ml/min. 1000 mg i.v.			1.6-2.1 (1.9)*		124-506 ml/min. (374 ml/min.)*	115-261 ml/min. (188 ml/min.)*		89.5-100 (95.6)*	van Dalen et al., 1979
RI ClCR<40 ml/min. 1000-1500 mg i.v.			4.4-17.4 (10.5)*		13-154 ml/min. (46 ml/min)*	3.5-138 ml/min. (34 ml/min.)*		13-88.7 (49.7)*	
H Cl_{CR}>60-120 ml/min./1.73 m^2 750 mg *i.v.*	72.0	5	1.7	0.19 l/kg	123	128	109	96	Bundtzen *et al.*, 1981
RI Cl_{CR}<20 ml/min./1.73 m^2 750 mg *i.v.*	66.0	5	17.6	0.27 l/kg	13	5.5	1070	45	

Health status: H: Healthy RI: Renal insufficiency **Cl_{CR}:** Creatinine clearance **Dose and route**	**Cmax** **(µg/ml)**	**Tmax** **(min.)**	**T1/2** **(h)**	**Vd** **(l/1.73 m2)**	**ClB** **(ml/min/ 1.73 m2)**	**ClR** **(ml/min/1.73 m2)**	**AUC** **(µg/ml/h)**	**24 h urinary excretion** **(% of dose)**	**Reference**
RI Cl_{CR}<20 ml/min. 750 mg *i.v.*	52.2-125		6.5-22.3 (12.4)*	11.9-29.6 l (18.1 l)*	15.3-22.9 ml/min (18 ml/min.)*	3.5-13.8 ml/min. (9 ml/min.)*		25-63 (46)*	Walstad *et al.*, 1983
H 500 mg *p.o.*	6.7	121	1.4	33.5 l			27.3	54.5 (during 8 hours)	Ishibiki *et al.*, 1990
RI Cl_{CR}<30 ml/min. 500 mg *i.v.*	48.7		12.6	22.8 l	22.3 ml/min.				Davies *et al.*, 1991
H Cl_{CR}>85 ml/min./1.73 m^2 500 mg *p.o.*	4.4	180	1.4				21.6	41.9	Konishi *et al.*, 1993
RI Cl_{CR}<15 ml/min./1.73 m^2 500 mg *p.o.*	9.2	240	16.8				258	17.5	
H 500 mg *p.o.*	5.2	147	1.3				19.9		Nix *et al.*, 1997
H 750 mg *p.o.*	6.5		1.5				52.4		Massias *et al.*, 1998

H - this also means that Cl_{CR} values were usually > 100 ml/min./1.73 m^2 or Cl_{CR} values were not specified

* - recalculated mean values

Assessment of the Sponsor response to Question 2

Assessment of the Sponsor response to Question 1 indicates that it is not possible to determine the identity of the products of the transformation of cefuroxime in bovine milk without specific experimental data.

The central point in the assessment of the toxicity of the residues of cefuroxime is whether the residues (metabolites) found in milk are the same compounds which are formed in animal species used for toxicity testing and in exposed humans, as those found in bovine milk. In the present case, however, the nature of metabolites and degradation products is not known and the data requested by the 58th JECFA have not been provided by the Sponsor. The additional interpretations in the newly submitted expert report are not suited to replace the required experimental data.

CONCLUSIONS AND RECOMMENDATION

Considering that:

- the information requested by the 58th JECFA has not been provided by the Sponsor;
- the nature of the products of the transformation of cefuroxime in bovine milk remains unknown;
- it is not possible to assess the toxicological significance of unknown cefuroxime residues in bovine milk;

the Committee decided not to extend the temporary ADI for cefuroxime and the temporary MRL for cefuroxim in bovine milk.

REFERENCES

Barnes A.R. (1990) Chemical stabilities of cefuroxime sodium and metronidazole in an admixture for intravenous infusion. J. Clin. Pharm. Ther. 15, 187-196.

Bundtzen R.W., Toothaker R.D., Nielson O.S., Madsen P.O., Welling P.G. and Craig W.A. (1981) Pharmacokinetics of cefuroxime in normal and impaired renal function: comparison of high-pressure liquid chromatography and microbiological assays. Antimicrob. Agents Chemother. 19, 443-449.

Chaudhary R.K., Srivastava A.K. and Rampal S. (2001) Effect of *E. coli* endotoxin induced fever on the pharmacokinetics and dosage regimen of cefuroxime in calves. Indian J. Pharmacol. 33, 425-430.

Coomber P.A., Jefferies J.P. and Woodford J.D. (1982) High-performance liquid chromatographic determination of cefuroxime. Analyst 107, 1451-1456.

Das Gupta V. and Stewart K.R. (1986) Stability of cefuroxime sodium in some aqueous buffered solutions and intravenous admixtures. J. Clin. Hosp. Pharm. 11, 47-54.

Davies S.P., Lacey L.F., Kox W.J. and Brown E.A. (1991) Pharmacokinetics of cefuroxime and ceftazidime in patients with acute renal failure treated by continuous arteriovenous haemodialysis. Nephrol. Dial. Transplant. 6, 971-976.

Erskine R.J., Wilson R.C., Tyler J.W., McClure K.A., Nelson R.S. and Spears H.J. (1995) Ceftiofur distribution in serum and milk from clinically normal cows and cows with experimental *Escherichia coli*-induced mastitis. Am. J. Vet. Res. 56, 481-485.

Ferguson E. and Batten P. (1996) [^{14}C]-Cefuroxime: residues following intramammary administration of Spectrazol Milking Cow to cattle. Corning Hazleton (Europe), Report number: 808/71-1011 (S8101-001-R). Sponsor submitted.

Foord R.D. (1976) Cefuroxime: human pharmacokinetics. Antimicrob. Agents Chemother. 9, 741-747.

Frederiksen M.C. and Bowsher D. (1983) Pharmacokinetics of cefuroxime. Am. J. Obstet. Gynecol. 145, 381-382.

Galanti L.M., Hecq J.-D., Vanbeckbergen D. and Jamart J. (1996) Long-term stability of cefuroxime and cefazolin sodium in intravenous infusions. J. Clin. Pharm. Ther. 21, 185-189.

Gáspár A., Andrási M. and Kardos S. (2002) Application ofcapillary zone electrophoresis to the analysis and to a stability study of cephalosporins. J. Chromatogr. B 775, 239-246.

Gower P.E. and Dash C.H. (1977) The pharmacokinetics of cefuroxime after intravenous injection. Eur. J. Clin. Pharmacol. 12, 221-227.

Ishibiki K., Inoue S., Suzuki F., Okumura K., Takeda K. and Toshimitsu Y. (1990) Investigation of adsorption, metabolism and excretion of cefuroxime axetil in volunteers of gastrectomized patients. Jpn. J. Antibiot. 43, 337-344.

JECFA (2002) Cefuroxime. In: Evaluation of certain veterinary drug residues in food. Fifty-eight report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No 911, 14-26.

Jones R.N. (1989) A review of cephalosporin metabolism: a lesson to be learned for future chemotherapy. Diagn. Microbiol. Infect. Dis. 12, 25-31.

Jorgensen J.H., Redding J.S. and Maher L.A. (1988) Influence of storage and susceptibility test conditions on stability and activity of LY163892 and four other cephalosporins. Antimicrob. Agents Chemother. 32, 1477-1480.

Konishi K., Suzuki H., Hayashi M. and Saruta T. (1993) Pharmacokinetics of cefuroxime axetil in patients with normal and impaired renal function. J. Antimicrob. Chemother. 31, 413-420.

Lecaillon J.B., Rouan M.C., Souppart C., Febvre N. and Juge F. (1982) Determination of cefsulodin, cefotiam, cephalexin, cefotaxime, desacetyl-cefotaxime, cefuroxime and cefroxadin in plasma and urine by high-performance liquid chromatography. J. Chromatogr. 228, 257-267.

MacNeil J.D. and Arnold D. (2002). Cefuroxime sodium. In: Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper, No 41/14, 1-13.

Massias L., Muller-Serieys C., Farinotti R. and Bergogne-Bérézin E. (1998) Pharmacokinetics of cefuroxime in healthy volunteers: an update. J. Antimicrob. Chemother. 42, 408-410.

Nakamura T., Hashimoto I., Sawada Y. and Mikami J. (1987) Clinical studies on cefuroxime axetil in acute mastitis. Jpn. J. Antibiot. 40, 340-348.

Nanbo T., Takaichi M., Mitsugi K., Okamura K., Tsuji H. and Fukuda I. (1979) Pharmacokinetics and metabolic study of cefuroxime in rats and dogs. Chemotherapy (Tokyo) 27(Suppl. 6), 91-103.

Nix D.E., Symonds W.T., Hyatt J.M., Wilton J.H., Teal M.A., Reidenberg P. and Affrime M.B. (1997) Comparative pharmacokinetics of oral ceftibuten, cefixime, cefaclor, and cefuroxime axetil in healthy volunteers. Pharmacotherapy 17, 121-125.

Okumura K., Tsuji H., Fukuda I., Takeda K., Kobayashi J. and Kato H. (1979) The absorption, biodistribution, metabolism and excretion of cefuroxime. Chemotherapy (Tokyo), 27 (Suppl. 6), 104-110.

Oldham G.B. and Andrews V. (1996) Control of microbial contamination in unpreserved eyedrops. Br. J. Ophthalmol. 80, 583-584.

Parker R.C. (2003) Cefuroxime MRLs. Expert Report responding to questions raised by the 58th JECFA, 1-18. Schering-Plough Animal Health. Sponsor submitted.

Parker R.C. (2004) Response to questions raised by JECFA Experts prior to the start of the 62nd JECFA meeting. Schering-Plough Animal Health. Sponsor submitted.

Sanders C.A. and Moore E.S. (1986) Liquid-chromatographic assay of cefuroxime in plasma. Clin. Chem. 1986, 32, 2109.

Schlesser V., Hecq J.-D., Vanbeckbergen D., Jamart J. and Galanti L. (2001) Interest of stability studies of three cephalosporins in intravenous solution by HPLC. In: Proceedings of the 18th Conference on Immunoanalysis and Biologic Specialities, 24-26 October Nantes, France.

Silley P. and Rudd A.P. (1986) Cefuroxime concentration in cattle serum following either on intramuscular injection of cefuroxime sodium suspension in glycerol formal or an intramuscular injection of cefuroxime sodium aqueous suspension. Glaxo Animal Health Limited, Report No. An.H.86/R/88. Sponsor submitted.

Soback S., Ziv G. and Kokue E.-I. (1989) Probenecid effect on cefuroxime pharmacokinetics in calves. J. Vet. Pharmacol. Ther. 12, 87-93.

The Merck Index (1989) Cefuroxime. Merck & Co., Inc. Rahway, NJ, USA.

van Dalen R., Vree T.B., Hafkenscheid J.C.M. and Gimbrère J.S.F. (1979) Determination of plasma and renal clearance of cefuroxime and its pharmacokinetics in renal insufficiency. J. Antimicrob. Chemother. 5, 281-292.

Vree T.B. and Hekster Y.A. (1990) Pharmacokinetics and tissue concentrations of cefuroxime. Pharm. Weekbl. Sci. 12, 262-267.

Walstad R.A., Nilsen O.G. and Berg K.J. (1983) Pharmacokinetics and clinical effects of cefuroxime in patients with severe renal insufficiency. Eur. J. Clin. Pharmacol. 24, 391-398.

Wang D. and Notari R.E. (1994) Cefuroxime hydrolysis kinetics and stability predictions in aqueous solution. J. Pharm. Sci. 83, 577-581.

ALPHA-CYPERMETHRIN and CYPERMETHRIN

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ADDENDUM

To the alpha-cypermethrin and cypermethrin monographs prepared by the 47th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/9, Rome 1997; the 54th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/13, Geneva 2000, for cypermethrin only; and the 58th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/14, Rome 2002

IDENTITY

Chemical names: Alphacypermethrin:

A racemate of (S)-alpha-cyano-3-phenoxybenzyl (lR,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate and (R)-alpha-cyano-3-phenoxybenzy1 (IS,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC name); and a racemate of (S)-alpha-cyano-3-phenoxybenzyl (lR)-cis-3- (2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate and (R)-alpha-cyano-3-phenoxybenzyl (1S)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate. (CAS No. 67375-30-8)

Cypermethrin:

(RS)-alpha-cyano-3-phenoxybenzyl-(1RS, 3RS, 1RS, 3RS)-3-(2, 2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC name); (RS)-cyano-(3-phenoxyphenyl)methyl(1RS)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate (CAS No. 52315-07-8)

Common names: FASTAC, CONCORD, FENDONA, RENEGADE. (alpha-cypermethrin)

Structural formula: See next page.

Molecular Formula: $C_{22}H_{19}C_{l2}NO_3$

Molecular weight: 416.3

Appearance: White-to-cream crystalline solid

Stability: Highly stable to light and elevated temperatures. It is resistant to acidic hydrolysis but undergoes ester cleavage in environmental (basic) aquatic conditions. Its low water solubility indicates a low bioavailability in aquatic situations.

Melting point: 81.4-84.0°C

Boiling point: 200°C at 9.31 PA

Octanol-water partition coefficient: p = 3.16x10s

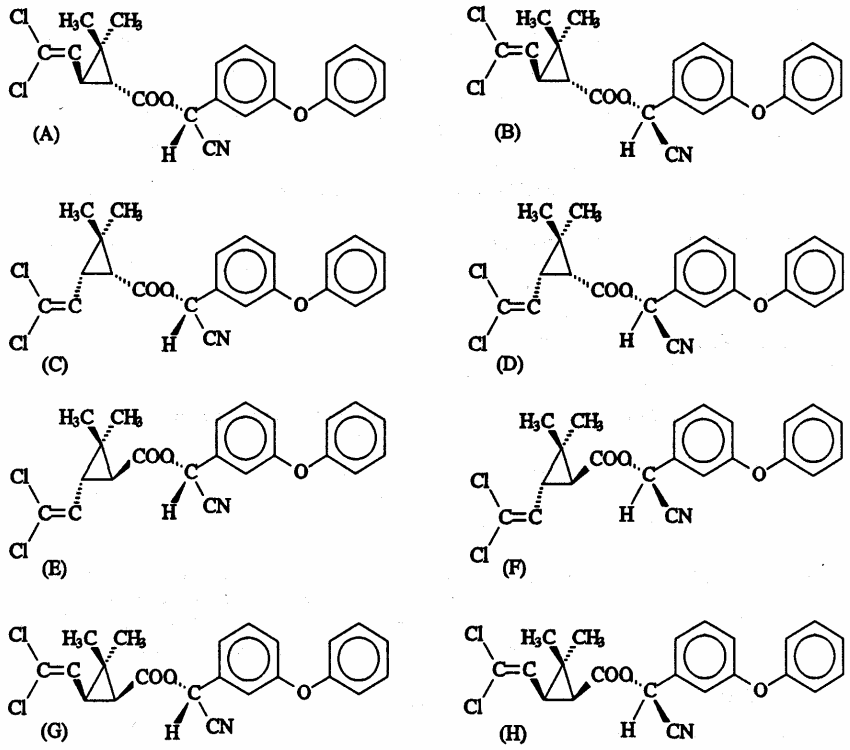
Density: 1.330 g/ml (typical for pure material)

Solubility (g/l at 21°C)

n-Hexane	6.5
Propanol-2	9.6
Methanol	21.3
Ethyl acetate	584
Toluene	596
Fat	78
Water	2.06 μg/l at 20°C

Alphacypermethrin was determined to be miscible with acetone and dichloromethane at room temperature

Structural formula:



Chemical structure of eight cypermethrin stereoisomers. Alphacypermethrin comprises the (D) and (G) isomers.

(A) (1R,*trans*) (αR); (B) (1R,*trans*) (αS); (C) (1R,*cis*) (αR); (D) (1R,*cis*) (αS); (E) (1S,*trans*) (αR); (F) (1S,*trans*) (αS); (G) (1S,*cis*) (αR); and (H) (1S,*cis*) (αS)

Alphacypermethrin Cis 2: (D) and (G) isomers
Alphacypermethrin Cis 1: (C) and (H) isomer
Cypermethrin is a mixture of all isomers

INTRODUCTION

Alpha-Cypermethrin

Alpha-Cypermethrin was first reviewed by the Committee at its forty-seventh meeting in 1996 (FNP 41/9). Temporary MRLs for cattle, sheep and poultry were recommended at the 47th meeting: 500µg/kg in fat, 100µg/kg in muscle, liver and kidney, 25µg/kg for cattle whole milk and 50µg/kg for eggs, expressed as parent drug. The temporary MRLs accommodate the ADI and the recommended use of alpha-cypermethrin as a veterinary drug: The theoretical maximum intake of residues would be 406µg per day, compatible with the maximum 1200 µg based upon the ADI of 0-20 µg/kg body weight. In reaching its decision on MRLs for alpha-cypermethrin, the Committee took the following factors into consideration:

- An ADI of 0-20 µg/kg of body weight was established, equivalent to a maximum theoretical daily intake of 0-1200 µg for a 60 kg person.
- The parent drug was identified as the marker residue.
- Fat, milk and eggs were target tissues.
- The metabolism of the two isomers forming alpha-cypermethrin was similar to that of the other six isomers in cypermethrin. There was certain evidence, but not fully demonstrated, that no interconversion of the cis forms to the trans forms took place during metabolism.
- The metabolism and radio depletion studies were insufficient. Metabolite profiles were not determined in sheep or poultry. Limited studies were presented for cattle.

- The relationship between the concentration of alpha-cypermethrin and total residues was imprecise. A very conservative estimate of parent drug as a percent of total residues in all food species was proposed: muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80 and eggs, 30. These percentages were the same as proposed for cypermethrin by the Committee (the racemic mixture of eight isomers includes the two that correspond to alpha-cypermethrin).
- Adequate residue information from non-radiolabelled studies using the recommended formulations was provided.
- For cypermethrin, bound residues were lower than 20 % in liver and 10 % in other tissues (FNP 41/9)
- Analytical methods were available but validation was needed.

At its 47th meeting the Committee requested the following new information:

1. Radiodepletion studies in sheep and poultry which extended beyond the recommended withdrawal time using the drug in its topical formulation. The study must determine the depletion of the total residues and the parent drug;
2. The radio depletion studies submitted for cattle should be reassessed to determine the depletion of total residues and the parent drug;
3. Evidence of lack of interconversion of the cis isomers to the trans isomers during metabolism in the target species; and
4. Further information on the validation of the analytical methods, particularly data on the derivation of LOD and LOQ.

Since the information required at the 47th meeting of the Committee was not provided at the 54th meeting of the Committee, the temporary MRLs for cattle, sheep and chicken were not extended. The Committee requested similar data to be provided for evaluation at the 58th meeting of the Committee.

Two new radiolabel studies were submitted for evaluation at the 58th meeting of the Committee– one in sheep and one in cattle. As the market for poultry did not support conducting additional work, studies for poultry were not carried out. One additional report was provided on analytical methods. All the studies were carried out using appropriate and applicable good laboratory practices.

Based on the data provided, the 58th meeting of the Committee recommended the following MRLs of alpha-cypermethrin in cattle and sheep tissues and cattle milk: muscle, liver and kidney, 100µg/kg; fat, 1000µg/kg and cattle milk, 100µg/kg. Maximum residue limits in cattle and sheep liver and kidney are based on the LOQs of the GC-ECD method (50µg/kg for cattle and 20µg/kg for sheep) as residues were less than the LOQs at all sampling times. MRLs in fat, muscle and cattle milk were based on residue data of studies submitted for evaluation.

Cypermethrin

Cypermethrin was first reviewed by the .Joint Meeting on Pesticide Residues (JMPR) in 1979 and subsequently in 1981, 1986, 1988 and 1990. MRLs were recommended for a wide range of crops, meat and milk products and feed commodities. Whereas cypermethrin has been used on horses, deer, goats and sheep, it was evaluated for use only on cattle, sheep and pou1try by the 47th meeting of the Committee. The 47th meeting of the Committee recommended temporary MRLs for cattle, sheep and poultry of 200 µg/kg in muscle, liver and kidney, 1000µg/kg in fat, 50 µg/kg for cattle whole milk and 100µg/kg for eggs expressed as the parent drug. The JMPR exposure intake calculations use approximately 300 µg for pesticide use, leaving 2700 µg for veterinary use. The theoretical maximum daily intake was 810µg for use as a veterinary drug. In reaching its decision, the Committee took the following factors into consideration:

The ADI was 0-50 µg/kg body weight established by JMPR (1981), equivalent to 0-3000µg for a 60 kg person. The marker residue is the parent drug, cypermethrin. Fat, milk and eggs are marker tissues

The metabolism and radio depletion studies were not adequate and, therefore, very conservative estimated of the marker compound as a percent of total residues in all food species was applied. The percentages proposed for the estimation in individual tissues of total residues from the parent drug were: muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80; and eggs, 30.

There is adequate information from the non-radiolabel studies using the recommended formulations.

There are analytical methods available, however, evidence of adequate validation was needed.

The 47th Committee requested the following information to further elaborate MRLs at the 54th meeting of the Committee:

1. Radiodep1etion studies that extend beyond the recommended withdrawal times and using the drug in its topical formulation. The study should determine the depletion of the total residues and the parent drug.
2. Evidence to verify the limited information concerning no-interconversion of isomeric forms during metabolism in the target species.
3. Further information on the validation of the analytical methods, particularly data on how the LOD and LOQ were determined.

The 54th meeting of the Committee considered a new radiolabelled study in sheep treated orally with a 80:20 cis:trans isomer ratio, not topically treated as requested. The Committee considered that no information was submitted to the first two requests so the temporary MRLs recommended for all animal tissues and milk were not extended. The Committee also noted that no information was made available for the toxicological evaluation of the 80:20 cis:trans cypermethrin. In answer to request 3, a

suitable analytical method to measure the sum of isomers in mixtures of cypermethrin by GC was submitted. For re-evaluation at the 58th meeting, the Committee requested similar data to be provided (items 1, 2 and 3).

The 47th meeting of the Committee only considered the 45:55 cis-trans cypermethrin mixture and the recommended use of cypermethrin as an ectoparasiticide. No toxicological evaluation was performed for the 80:20 cypermethrin isomeric mixture. The 58th meeting of the Committee considered a conservative approach, using the toxicology assessment of alpha-cypermethrin (100% cis isomers). An ADI of 0-20µg/kg body weight was established at the 47th meeting of the Committee for alpha-cypermethrin and used for the 80:20 cis-trans isomer cypermethrin product.

Based upon the new information provided, the 58th meeting of the Committee recommended MRLs in sheep, measured as cypermethrin equivalents of: 20µg/kg muscle, liver and kidney and 200µg/kg for fat.The MRLs in muscle, liver and kidney were recommended using the limit of quantitation of the method (10µg/kg) as residues at 7 days post-treatment are above the LOQ only in fat tissue. MRLs for fat were based on the residue studies using a pour-on formulation reported at the 54th Committee.

The theoretical maximum daily intake accounts for approximately 6 percent of the alpha-cypermethrin ADI. As the sponsor did not indicate support for MRLs in species other than sheep, the temporary MRLs in cattle and poultry were not retained.

The Joint Meeting on Pesticide Residues plans to review cypermethrin in 2005.

The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Food (CC/RVDF) considered the recommendations of the Committee (Codex Alimentarius Commission, Alinorm 03/31A, 2003) and decided to retain the recommended MRLs from the 58th meeting of the Committee at Step 4 in view of concerns expressed on the elaboration of separate MRLs for both compounds and requested that JECFA consider the establishment of one ADI and one set of MRLs for the entire cypermethrin group.

RESIDUES IN FOOD AND THEIR EVALUATION

General

Alpha-cypermethrin and cypermethrin are highly active synthetic pyrethroid insecticides which are effective against a wide range of pests relevant to public health and animal husbandry.

Alpha-cypermethrin consists of two of the four cis isomers present in cypermethrin. These isomers are the most biologically active enantiomeric pair. It is used in veterinary medicine for the control of ectoparasites such as ticks, fleas, lice and blowflies (EMEA, 1998).

Cypermethrin consists of a mixture of 4 cis- and 4 trans-isomers. The cis isomers are more acutely toxic than the trans isomers. It may be used, as a pesticide in a 45:55 cis:trans formulation or as a veterinary drug in two formulations, either 45:55 cis:trans or 80:20 cis:trans, named high cis cypermethrin (HCC). Cypermethrin commercial formulations for cattle and sheep are available as ear tags, sprays, dips and pour-on formulations (12.5mg /kg, 0.72-0.75g/animal)

Previous studies on alpha-cypermethrin

Dosage

Alpha-cypermethrin is applied as pour-on preparation for cattle (15 g/L, 0.15-0.75 g/animal) and sheep (2.5 g/L, 0.3-0.5 g/animal) and also as a dip for sheep and as a spray for poultry (10 mg/animal) (EMEA, 1998)

Radiolabelled drug studies in cattle

One lactating cow was dosed orally twice daily for 8 days, (0.25 g/day) with ^{14}C- alpha-cypermethrin. Of the administered dose, 58% was recovered (34% in faeces, 23% in urine, <1% milk) (Morrison and Richardson, 1994). Residue amounts were 390-480µg/kg for fat, 19-29µg/kg for muscle, 560µg/kg for liver, 220µg/kg for kidney and less or equal to 200µg/kg for milk. (FNP 41/9, pg.62 , table 1).

In another study four cows were dosed with 0.15 g pour-on treatment and sampled at 7, 14, 28 and 35 days post-dose (Redgrave et al, 1992). Total radiolabelled residues were mostly below the limit of quantitation (LOQ =10-30µg/kg) with the exception of some samples of fat (maximum 30µg/kg). Radiocounting estimates were not different from levels of alpha-cypermethrin measured by GC-ECD analysis (LOQ = 10ug/kg). In milk, maximum levels reached 7µg/kg by day 2-3 (71% determined as alpha-cypermethrin by GC), falling to the LOD for radiocounting (1µg/kg) by day 7. (FNP 41/9, pg 62, reference study 2, table 1)

In a third study (FNP 41/14, pg 23-36), ^{14}C-alpha-cypermethrin formulated at a nominal concentration of 15 g/L was topically administered to 8 steers (140-190 kg bw) and 8 lactating cows (510-560 kg) along the region of the back between shoulders and rump along the mid-dorsal line. Cows were treated following the morning milking. Mean doses achieved were 3 mg/kg body-weighth. Two steers and two cows were sampled at each time point. The total radiolabelled residues and extractability of different tissues were measured using a radiometric method for tissues and direct liquid scintillation counting for milk. Results are summarized in Table 1.

Table 1. Depletion of ^{14}C-alpha-cypermethrin in tissues and milk of cattle (mg/kg equivalents mean concentration ±std dev)

Tissue/Time	Post treatment time			
	3 day	7 day	14 day	21 day
Back fat	0.08 ±0.05	0.34 ±0.34	0.17 ± .03	0.65 ±0.07
Omental fat	0.05 ±0.04	0.20 ±0.14	0.28 ±0.12	0.31 ±0.12
Liver	0.08 ±0.05	0.18 ±0.08	0.17 ±0.06	0.10 ± .05
Kidney	0.03 ±0.03	0.04 ±0.02	0.06 ± .01	0.03 ± .02
Muscle*	<0.01(NA)	0.01 ±0.02	0.00 ± .00	0.01 ±0.00
Milk	0.04 ±0.03	0.02 ±0.01	0.00 ±0.00	0.00 (NA)

NA= not applicable
Data expressed as mean (n=4) ± SD in tissue, number of milk samples is variable
* Many results were calculated from data less than 30 dpm above background

^{14}C-total residues were detected at all times points post treatment in all tissues. The maximum ^{14}C alpha-cypermethrin residues as analyzed by HPLC radio-analysis were <35µg/kg in kidney and muscle, 647µg/kg in back fat and 421µg/kg in omental fat and 83µg/kg in milk. No ^{14}C-alpha-cypermethrin was detected in liver tissue. The percent parent drug to total residues at different time points were: 84 ±11% for back fat, 91±10% for omental fat, 90% in muscle (only one sample) and 16±13% for kidney and 96±23% in milk.

Using a GC-ECD method of analysis following the topical treatment noted above, alpha-cypermethrin residues followed the same tendencies with time as the radiolabel measures and also in milk at different milking times (60-126 h, n=13). The GC-ECD maximum results were: <50µg/kg for kidney, muscle and liver, 713µg/kg for back fat, and 337µg/kg for omental fat and 89µg/kg for milk. The percent of alpha-cypermethrin to TRR calculated from GC-ECD analyses of alpha-cypermethrin were lower than from studies using HPLC radio analysis: 76±44%, 70±16% and 76±13% in back fat, omental fat and milk, respectively. The results are summarized in Table 2.

Table 2. Maximum concentration of alpha-cypermethrin residues in cattle tissues following topical treatment.

	Post treatment (days)	Tissue	α-Cypermethrin µg/kg	Ratio (α-Cyper/total residues)
HPLC-radioanalysis study	21	Back fat	647	84
	21	Omental fat	421	91
	14	Kidney	22 (<35)	16
	7	Muscle	35	90
		Liver	ND.	
	60h	Milk	83	86
CG-ECD study	21	Back fat	713	76
	21	Omental fat	337	70
	All times post treatement	Kidney	< LOQ *	ND
		Muscle		
		Liver		
	60h	Milk	89	76

Note: ND=non detected . *LOQ tissues= 50µg/kg
References: Table 3 and Table 4, FNP 41/14, pg 23-36

The metabolite profiles in tissues and in cattle milk were defined. The ratio of parent drug to total ^{14}C- radiolabelled residues (TRR) in edible tissues was investigated at various withdrawal periods. In cattle, depletion rates of alpha-cypermethrin residues in edible tissues from steers and lactating cows were provided beyond the recommended 14 day withdrawal time for tissues and 0 day for milk following a single topical application.

The identified metabolites indicated that alpha-cypermethrin in steers and lactating cows, following topical application, underwent phase I oxidative hydroxylation at the phenyl ring and hydrolysis at the ester linkage to finally produce 3-phenoxybenzoic acid and its conjugates. The ester hydrolysis products were further oxidized to form 3-phenoxybenzoic acid (3- PBA) and 4-hydroxy-3-phenoxybenzoic acid (4'-OH-3-PBA). These compounds contained a free carboxylic and hydroxyl moiety, respectively, that underwent phase II metabolism to form glutamic acid and sulfate conjugates. Thus, the metabolism of alpha-cypermethrin was the same in orally treated cows (Morrison and Richardson 1994) and rat (Crawford and Hutson, 1977). Conversion from cis to trans configuration did not occur in the milk or steer tissues.

Identification and quantification of extracted radiolabelled residues showed that alpha-cypermethrin as the cis isomeric form was the main metabolite in both omental and back fat, milk and in the only sample of muscle analyzed. Extensive metabolism was also shown in liver and kidney, where the main metabolite identified was 3-PBA glutamate. Others metabolites tentatively identified were 3-PBA, 4-OH-3-PBA and 3-PBA-4-O-sulfate. A number of unknown extractable residues and bound residues were also detected in liver and kidney.

Two analytical methods for the determination of alpha-cypermethrin residues in cattle tissues (muscle, fat, kidney and liver) and milk were reported, bearing the titles SAMS 456-1 and SAMS 461-1, respectively. They were validated for determination of the LOQ values reported. They proved to be suitable for determination of alpha-cypermethrin in fat tissues of cattle and milk of cattle (LOQ = 50 µg/kg for cattle tissues, 10 µg/kg for cattle milk). However, because of the low residue concentrations in muscle, liver and kidney, the methods were of limited value for determination of residues in these tissues (most values were below the LOQ). The analysis of the chromatograms suggested that lower LOQs might be possible.

Radiolabelled drug studies in sheep

A sheep study extending beyond the recommended withdrawal time of 7 days for the pour-on formulation was reviewed by the 58th meeting of the Committee. Alpha-cypermethrin was formulated at a nominal concentration of 12.5 g/l and was topically administered on either side of the spine and around the rump to 6 male and 6 female sheep (28-39 kg BW) at a dose level of 15 mg/kg BW. This was the maximum recommended dose. (FNP 41/14, pg 23-36). The metabolite profile in tissues and in cattle milk was defined using only two samples (one ram and one ewe). The ratio of parent drug to total ^{14}C- radiolabelled residues (TRR) in edible tissues was investigated at various withdrawal periods. The total ^{14}C- radiolabelled residues (TRR) were determined using a radiometric method in tissues. For fat, residues were by direct liquid scintillation counting (LSC) and for other tissues, LSC following combustion. Results are summarized in Table 3.

Table 3. Depletion of ^{14}C-alpha-cypermethrin (mean concentration, mg/kg equivalents±std dev) in sheep tissues

Tissue/Time	Post treatment time			
	2 day	4 day	7 day	14 day
Back fat	0.62 ±0.74	0.66 ±0.70	0.17 ±0.06	0.08 ±0.03
Omental fat	0.11 ±0.06	0.23 ±0.01	0.19 ±0.12	0.14 ±0.06
Liver	0.04 ±0.02	0.08 ±0.05	0.04 ±0.02	0.02 ±0.00
Kidney	0.07 ± 0.03	0.14 ±0.06	0.06 ±0.02	0.02 ±0.01
Muscle	0.02 ±0.01	0.01 ±0.01	0.01 ±0.00	0.01 ±0.00

Data expressed as the mean (n=3 ±SD)

The maximum ^{14}C alpha-cypermethrin residues as analyzed by HPLC radio-assay were 1323µg/kg for back fat, 314µg/kg for omental fat, 22µg/kg for kidney and <20µg/kg for muscle and liver . The percentage of parent drug to total residues at different time points were 85 ±5% for back fat, 83±17% for omental fat and 62 ±23% in muscle, 9±6% in liver and 6 ±8% in kidney.

Using a GC-ECD method, alpha-cypermethrin residues followed the same tendencies with time as in the radiolabel study. The GC-ECD maximum levels were: 1360 µg /kg for back fat, 218 µg /kg for omental fat, and <20µg/kg for muscle, kidney and liver. The percentage of alpha-cypermethrin to TRR calculated from GC-ECD analyses of alpha-cypermethrin were lower than results from HPLC radioanalysis, 85±20% for back fat and 59±18% for omental fat. These results are summarized in Table 4.

Table 4. Maximum alpha-cypermethrin residues in sheep tissues from topical treatment.

	Post treatment (days)	Tissue	α-Cypermethrin µg/kg	Ratio (α-Cyper/total residues)
HPLC-radioanalysis study	2	Back fat	1323	85
	7	Omental fat	314	83
	2	Kidney	22	6
	2	Muscle	18	62
	2	Liver	10	9
CG-ECD study	4	Back fat	1360	85
	7	Omental fat	218	59
	All times post treatement	Kidney	< LOQ *	ND
		Muscle		
		Liver		

ND=non detected . *LOQ tissues= 20µg/kg
Reference: Table 8, FNP 41/14, pg 23-36

The metabolic fate of alpha-cypermethrin was the same as found in steers. Interconversion of the cis to trans isomeric form of alpha-cypermethrin was not observed. The cis isomeric form of alpha-cypermethrin was the main residue in fat tissues and

muscle. The main metabolite in liver was the 4-OH-parent and in kidney was 3-PBA-glutamate. A number of unknown extractable residues and bound residues were also detected.

Other residue depletion studies (with unlabelled drug) in cattle

Fifteen calves were dosed with 0.16g per animal as a pour-on treatment (Sherren, 1988b). Sampling time of residues were 3, 7 and 14 days post-dose. Maximum residues in both subcutaneous and perirenal fat occurred at day 7 (80-270 µg /kg). Residues were detected in kidney (<30 µg /kg), but were not detectable in muscle and liver (LOQ = 10µg /kg) (FNP 41/9, pg 64, reference study 2, table 2).

In a second study, two groups of 11 female calves were dosed at 0.15g pour-on treatment per animal (Cameron et al., 1993). Residue sampling times were 3, 7, 14, 21 and 28 days post-dose. Maximum residues in both subcutaneous and perirenal fat occurred at day 14 (20-100 µg /kg), then declined until day 28 (<40µg/kg) (FNP 41/9, pg 64, reference study 3, table 2). In both studies residues were higher in perirenal fat than in subcutaneous fat.

In another study 15 cows (five per treatment group) were treated at 0.1, 0.15 and 0.2g per cow (Sherren, 1988a). Tissues were sampled at 1, 2, 3, 4, 7, 14 and 21 days post-dose. Maximum residues (up to 5 µg /kg) were observed between days 2-5 after treatment and were all less than the LOQ (2 µg /kg) by day 21 for all treatments. The residue profile follows closely that seen with the radiolabelled study using the 0.15 g dose (FNP 41/9, pg 64, reference study 1, table 2).

Similar residue profiles to those obtained in the radiolabelled studies are found, showing residues principally in fat tissues (perirenal higher than subcutaneous), followed by kidney and minor quantities in muscle. Maximum residues in fat tissues occurred between 7 and 14 days at the same pour-on dose. Results were not corrected for recovery although they were determined. In milk, maximum residues were generally observed at short times after treatment (2-5 days) declining thereafter. Analytical data suggests that the residues were mainly alpha-cypermethrin. Most measurements were near the LOQ of methods employed.

Other residue depletion studies (with unlabelled drug) in sheep

Six sheep, three treated with a pour-on and three dip-treated, dosed at 0.2 g pour-on and 60 mg/l dip, were analyzed for residues in fat, skin and wool at 3, 7 and 14 days post-dose (Francis and Gill,1989). High residues were found in skin (up to 1400 ug/kg) for at least two weeks in both treatments. Subcutaneous fat residues were not detectable within 7 days of dosing in the pour-on treatment, but in dip treated sheep, residues were 40 ug/kg at 7 and 14 days of dosing (minimum concentration measured was 10 ug/kg) (FNP 41/9, pg 64, reference study 4, table 2).

Ten sheep treated with a pour-on formulation (five dosed at 0.01 g/kg bw and five at 0.02 g/kg bw), were sampled at 7 days post-dose (White, 1987). Residues after treatment in both perirenal and omental fat were 0.2-8 ug/kg and 3-11 ug/kg, respectively, at the 0.1 g/kg bw treatment and 5-18 ug/kg and 2-19 ug/kg, respectively, at the 0.2 g/kg bw treatment (FNP 41/9, pg 64, reference study 5, table 2).

In these studies, residues were measured only in fat and uncorrected for recovery. Others tissue residues were not measured. The majority of residues seemed remained unabsorbed (high concentrations in skin and wool) for the external treatments. Bound residues were less than 20% in liver and 10% in other tissues (FNP 41/9, pg 53).

Previous studies on cypermethrin

Radiolabelled drug studies in sheep

Two male sheep were topically treated (21.9 mg/kg BW) and a third was treated orally (3.9 mg/kg BW) with ^{14}C-cypermethrin cis:trans 45:55 isomer mixture (Crawford and Hutson, 1977b) (FNP 41/9, pg 42). Tissues were extracted and analyzed using gas chromatography for cypermethrin. In the oral treatment, maximum TRR concentrations were 390, 360 and 410 µg /kg in liver, kidney and renal fat, respectivley, at day 2 after treatment. The percent of total residues attributable to cypermethrin was 65%, 8%, <1% and 33% in fat, liver, kidney and muscle, respectively. In the pour-on treatment, TRR were higher in fat tissues: 170-300 µg/kg and up to 3300-100000 µg/kg in subcutaneous fat at the site of application. Residues in liver, kidney and muscle were 100-140 µg/kg, 140-120 µg/kg and 30-60 µg/kg respectivelly, between 1 and 6 days post treatment. Percent of total residues attributable to cypermethrin were between 80-92%, 13-17% and <4% in fat, liver and kidney, respectively. In muscle, cypermethrin was not quantifiable. Results of these previous studies are summarized in Tables 5 and 6.

Table 5. Percent cypermethrin of total residues following treatment with ^{14}C-cypermethrin in sheep.

Tissue	Topical (24 h)	Topical (6 d)	Oral (2 d)
Liver	13	17	8
Kidney	< 3	< 4	<1
Muscle	NQ	NQ	33
Renal fat	88	80	63
Subcutaneous fat	-	92	67

NQ = non quantifiable

Table 6. Total residues (ug/kg equivalents) of ^{14}C-cypermethrin in sheep

Treatment	Time Post Treatment	Muscle	Liver	Kidney	Renal fat	Subcutaneous fat
Topical	1 day	30-40	100	140	170	100000*
	6 day	30-60	140	120	300	3300*
Oral	2 day	30-40	390	360	410	260

A study investigating the radiodepletion of a mixture of 80:20 cis:trans ^{14}C-cypermethrin administered orally (1 mg/kg BW) to adult sheep was submitted (FNP 41/13 pg 19). Three groups of five sheep (two sexes) were slaughtered at 1, 3 and 5 days after dosing. Both radiolabelled cis and trans cypermethrin were measured by radio-TLC only at the 1-day time point due to small quantities in later post treatment times. No residues of the trans isomer were detected. Maximum concentrations of TRR reached 334, 408 and 50µg/kg in liver, kidney and fat, respectively, at day 1 after treatment. The percent of total residues attributable to cypermethrin was 86%, 4%, 1.2% and 22% in fat, liver, kidney and muscle. See Table 7.

Table 7. Concentration of total residues and marker residue (ug/kg) in sheep 1 day post treatment following oral treatment with 14C-cypermethrin (1 mg/kg BW) using an 80:20 isomer mixture.

Tissue	TRR	cis-Cyp	trans-Cyp	Ratio (%) cyp:TRR
Liver	334 ± 23	13 ± 5	0	4
Kidney	408 ± 105	5 ± 1	0	1.2
Muscle	13± 3	3 ± 2	0	22
Fat	50 ± 13	43 ± 17	0	86

Other Residue Depletion Studies (with unlabelled drug)

Residue information was provided using dip and pour-on preparations (FNP 41/9, pg 42). The main residue measured was the parent compound, cypermethrin, determined by GC-ECD with non-validated methods. In sheep following a dip treatment, residues were close or below the LOQ in most cases for all tisúes. Residues were only found in perirenal and omental fat. In one of the pour-on studies, 20 sheep were treated with 0.375 g of cypermethrin and 20 sheep with 0.75g. Residues of cypermethrin reached maximum values of 40 µg/kg at 3-7 days after treatment, descending to 20 µg /kg at 28 days after treatment in both perirenal and omental fat. In a second study, 10 sheep were treated with 0.375 g of cypermethrin in two different pour-on formulations. Residues at 7 days post treatment were 18-35 µg /kg in omental fat and 4-10 µg /kg in perirenal fat (very low recoveries). Residues in subcutaneous fat were not measured.

In another study, **t**wenty four wethers were dunked into a dip containing 0.01% cypermethrin (FNP 41/13, pg 23). Residues were detected in omental fat, perirenal fat and muscle from <10 µg /kg (0 day) up to 170 µg /kg in perirenal fat at day 14. Residues could not be detected in liver and kidney.

A study with Merino ewes treated using a 2.5% cypermethrin pour-on at 15 ml (normal maximun dosa rate) and 30 ml (FNP 41/13, pg 23) was reported. For the recommended maximum dose rate of 15 ml, residues in both omental and perirenal fats reached peak values of 40 µg/kg after 7 days. Values for a double dose rate of 30 ml also peaked after 7 days at 70µg/kg for omental fat and 80 µg/kg for perirenal fat. For muscle, liver and kidney samples, results were all less than 20 µg /kg.

Forty two female Suffolk cross sheep (approximately 50-60 kg body weight and 9 months old) were treated with cypermethrin at a rate of 1 ml/kg BW (12.5 mg/kg; mean dose level 0.72-0.75 g/animal). The drug was applied by pin-stream application to the backline, directly onto the skin. Groups of five sheep were sacrificed at 7, 14, 21, 28, 35 and 42 days. Duplicate samples of liver, muscle, kidney and subcutaneous fat were taken from each animal and analyzed for cis-cypermethrin. The remaining two slaughter groups were not required for analysis. Analysis for muscle and kidney samples were stopped at 14 days post-treatment because residues were below the limit of quantitation or not detected in all samples at 7 and 14 days post treatment. Similarly, liver sample analysis was stopped at 21 days post treatment. Analysis of subcutaneous fat samples was terminated at 28 days post treatment. The limit of quantitation (LOQ) was 10µg /kg and the limit of detection (LOD) was 4µg /kg. To estimate mean values, analytical results below the LOQ and LOD were allocated values of half the LOQ. Results are presented in Table 8 and 9.

Table 8. Cypermethrin residues in Suffolk sheep following topical treatment at 12.5 mg/kg body weight

Post treatment (days)	Liver	Kidney	Muscle	Fat (µg /kg)
7	5 <LOD	2 <LOQ, 3 <LOD	3 <LOQ, 2 <LOD	33.9, 17.2, 25.8, 20.2, 36.2
14	3 <LOD, 2 <LOD	5 <LOD	5 <LOD	17.1, 17.9, 1 <LOQ, 2 <LOD
21	1 <LOD, 4 <LOD	NA	NA	5 <LOD
28	NA	NA	NA	5 <LOD

NA= samples not analyzed, previous analysis showed levels of BLQ or ND for two consecutive timepoints.

Table 9. Estimated cis-cypermethrin residues (ug/kg) in sheep tissues after topical treatment (12.5 mg/kg body weight)

Days		Liver	Kidney	Muscle	Subcutaneous Fat
7	Max	2.0	5.0	5.0	36.2
	Mean	2.0	3.2	3.8	26.7
	S.D.	2.0	1.6	1.6	8.3
14	Max	5.0	2.0	2.0	17.9
	Mean	3.8	2.0	2.0	8.8
	S.D.	1.6	0.0	0.0	8.0
21	Max	5.0	NA	NA	2.0
	Mean	2.6	NA	NA	2.0
	S.D.	1.3	NA	NA	0.0
28	Max	NA	NA	NA	2.0
	Mean	NA	NA	NA	2.0
	S.D.	NA	NA	NA	0.0

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Three analytical methods for the determination of cypermethrin residues in cattle tissues (MCY/01/51), in cattle milk (MC/01/50) and in sheep tissues (MCY/99/31) were submitted. The submission also enclosed supplements to the method reference MCY/99/31 (the stability of HCC in sheep kidney and LOD determination in sheep liver and muscle). The methods have been properly validated.

Determination of high cis-cypermethrin (HCC) in cattle tissue using GC-ECD (Method Reference MCY/01/51)

This method describes the determination of the concentration of HCC cypermethrin present in cattle tissues using capillary gas chromatography with on column injection and electron capture detection, following solid phase extraction.

Principle: The analyte was extracted from fat, kidney and muscle with acetonitrile whereas from liver, chloroform is the indicated extraction solvent. HCC residues were extracted from kidney, fat and muscle (0.9-1.1g) by homogenisation with acetonitrile and anhydrous sodium sulphate twice and organic phases separated. This combined extract was partitioned with hexane twice, the hexane being discarded. After centrifugation, the supernatant was decanted and evaporated to dryness under nitrogen. Same procedure was employed for liver using chloroform rather than acetonitrile. The dry residues from all tissues were reconstituted in hexane and further cleaned up on a FL Isolute SPE cartridge. The eluate was evaporated to dryness and redissolved in hexane. The final analysis was carried out using capillary gas chromatography with electron capture detection. The analyte eluted with ethyl acetate: hexane 10:90

Standards: The differing MRL values (those established by EMEA) for cattle fat and others tissues dictated the level of dilution of the stock standard solutions for the particular tissue being analysed. The nominal concentration of working standard and the nominal concentration of analytical quality control (AQC) standard were 200 ng/ml and 2000 ng/ml respectively for fat and 20 ng/ml and 200 ng/ml respectively for muscle, liver and kidney. Tissue blank samples were prepared as the test tissue samples but using tissue free of HCC. AQC standards were prepared as for the test tissue samples but using blank tissue sample fortified with 100 µl of the appropriate spiking solution. Residues of HCC are determined by GC with ECD detection (2µl injection volume). GC was performed in a SGE BPI column (15m x 0.53 mm ID, 1.0 µm) with a temperature gradient.

Linearity: The linearity of detector response was determined over the range of approximately 10 ng/ml to 400 ng/ml, with a correlation coefficient of 0.9979.

Accuracy and Precision: To establish the accuracy and repeatability, three fortification concentrations levels (MRL, 1/2x MRL and 2x MRL) were used for recovery determinations on day 1 (n=6 at each fortification level). Three more fortified tissue samples were analysed, at each of the above concentration levels, on Day 2 and Day 3 and involving more than one operator in order to determine the within laboratory reproducibility part of precision.

For fat, recoveries and coefficient of variation (CV) at each respective level (100, 200 and 400 µg/kg) were: 91.1% (5.1), 82.8% (4.1), 76.8% (7.5), respectively. Interday variation of fat analysis had a mean CV of 8.62% (n=12). For liver, recoveries and coefficient of variation (CV) at each respective level (10, 20 and 40 µg/kg) were: 79.5% (9.9), 82.8% (6.1), 77.9% (3.5), respectively. Interday variation of liver analysis had a mean CV of 9.36 % (n=12). Similarly, for kidney recoveries at the same levels were 88.7% (7.9), 83.0% (3.2) and 77.4% (7.7), respectively. Interday variation of kidney analysis had a mean CV of 9.36 % (n=12). For muscle, recoveries at the same levels were 89.3 % (4.2), 81.1% (4.7) and 78.7% (8.1), respectively. Interday variation of muscle analysis had a mean CV of 10.6 % (n=12).

Specificity: A blank tissue extract was analysed together with three tissue extracts spiked with the five compounds most likely associated with typical cattle tissue samples (ivermectin, moxydectin, permethrin, 3-phenoxybenzaldehyde and DCVC acid). The tissue extracts were spiked with 2x MRL of the compounds together with HCC at MRL level. No significant interference was detected and the percentage recoveries obtained for HCC were within the acceptable limits.

Stability: The stability of HCC in the final tissue hexane extracts was assessed by spiking cattle tissue with HCC at MRL level and then following the extraction procedure. The extract was stored under ambient conditions of temperature and light and analysed initially to a maximum of 6 days in the four tissues. The final hexane extracts are stable at least 5-6 days under the mentioned conditions.

The stability of HCC in cattle tissue, when stored at -23ºC± 3ºC, was determined by spiking blank tissue samples with HCC at MRL level, samples then stored frozen and analysed at set intervals. The data indicated that analyte was stable in fat, kidney and muscle for at least 4 weeks and in liver for at least 2 weeks. The sponsor claims that stock analytical standard solutions and the subsequent dilutions in hexane were stable for up 29-30 days from a previous method.

Limit of quantitation (LOQ): This was defined as the lowest level at which precision and accuracy have been determined, nominally 10µg/kg in liver, muscle and kidney and 100µg/kg in fat. Limit of Detection (LOD): This was calculated as the mean background level at the retention time of HCC plus 3x SD of the mean from the analysis of 20 independently extracted blank samples of tissues. For fat, liver, kidney and muscle the LODs were 5µg/kg, 5µg/kg, 2µg/kg, 2µg/kg and 2µg/kg, respectively.

Practicability: The analyses were performed using commercially available reagents and equipments. Sponsors declared that the method was performed safely by a trained analyst and a large number of samples were analysed in a reasonable time period.

Note: raw data presented by sponsor included chromatograms for cattle tissues blanks, an HCC standard at the MRL corresponding to each tissue, all tissues spiked with HCC at the respective LOQs and cattle tissues (kidney, muscle and fat) with HCC standard at the MRL in the presence of possible interfering compounds.

Determination of high cis-cypermethrin (HCC) in cattle milk by GC-ECD (Method Reference MCY/01/50)

The principle of this method is the same as in the method described before, differing in two points: in this case,the solvent used in the first extraction was acetonitrile and in the solid phase extraction, florisil cartridges were used. Standard, milk blank samples and AQC standards were prepared in identical way to the previous described method.

Linearity: The linearity of detector response was determined over the range of approximately 10 ng/ml to 400 ng/ml, with a correlation coefficient of 0.9979.

Accuracy and precision: To establish the accuracy and repeatability, three fortification concentrations levels (MRL, 1/2x MRL and 2x MRL) were used for recovery determinations on day 1 (n=6 at each fortification level). Three more fortified milk samples were analysed, at each of the above concentration levels, on Day 2 and Day 3 and involving more than one operator in order to determine the within laboratory reproducibility part of precision. Mean recoveries and coefficient of variation (CV) at each respective level (10, 20 and 40 µg/l) were 79.9% (3.6), 79.3% (4.5), 71.2% (3.9), respectively. Interday variation of milk analysis had a mean CV of 5.6% (n=12).

Specificity: Blank milk extracts were spiked with solutions containing the five compounds most likely associated with typical cattle milk samples (ivermectin, moxydectin, permethrin, 3-phenoxybenzaldehyde and DCVC acid). The tissue extracts were spiked with 2x MRL (40µg/kg) of the compounds together with HCC at MRL level (20µg/kgl) No significant interference was detected and the percentage recoveries obtained for HCC were within the acceptable limits.

Stability: The stability of HCC in the final hexane extracts was demonstrated for up to 7 days at ambient temperature. The stability of HCC in cattle milk, when stored at -23ºC±3ºC, was demonstrated for up to 13 days. The sponsor declared that stock analytical standard solutions and the subsequent dilutions in hexane were stable for up 29-30 days from a previous method.

Limit of quantitation (LOQ): This was defined as the lowest level at which precision and accuracy have been determined, nominally 10µg/kg. Limit of determination (LOD): This was calculated as the mean background level at the retention time of

HCC plus 3x SD of the mean from the analysis of 21 independently extracted blank samples of milk. The rounded up calculated LOD was 6µg/l.

Practicability: The analysis was performed using commercially available reagents and equipments. Sponsors declared that the method was performed safely by a trained analyst and a large number of samples were analysed in a reasonable time period. The sponsor provided the following chromatograms: a typical cattle milk blank, a typical HCC standard spiked at the MRL level (20µg/kgl), cattle milk spiked with HCC at the LOQ (10µg/kg) and HCC standard in the presence of possible interfering compounds

Determination of High Cis Cypermethrin (HCC) in sheep tissue using GC-ECD (Method Reference MCY/99/31)

The principle of this method is the same as in the previous described methods, differing in solvents of the first extraction: chloroform was used for liver, kidney and muscle and acetonitrile for fat. Standard, milk blank samples and AQC standards were prepared in identical way to the previous described methods.

Linearity: The linearity of detector response was determined over the range of approximately 10 ng/ml to 400 ng/ml, with a correlation coefficient of 0.998.

Specificity: A blank tissue extract was analysed together with tissue extracts from fat and kidney spiked with the two compounds most likely associated with typical cattle tissue samples (propetamphos and abarmectin). The tissue extracts were spiked with 20ng/ml and 40 ng/ml, but no significant interference was detected.

Limit of quantitation (LOQ): This was defined as the lowest level at which precision and accuracy have been determined, nominally 10µg/kg in the four tissues.

Note: raw data presented by sponsor included chromatograms for kidney and fat tissue blanks, an HCC calibration standard, all tissues spiked with HCC at the LOQs and sheep kidney and fat with HCC standard in the presence of possible interfering compounds.

Addendum I to the method reference MCY/99/31

The results of a study about the stability of HCC in sheep kidney were provided (raw data were not submitted). The stability in sheep kidney tissue, when stored at -23ºC±3ºC, was determined by spiking blank tissue samples with HCC at MRL level (20µg/kg), samples then stored frozen and analysed at set intervals. The measured percentage recovery appeared to drop off rapidly after 11 days (under 65%) and hence 11 days was chosen as the maximum period of stability under the stated storage conditions.

Addendum II to the method reference MCY/99/31

The information submitted complement the original report of the method reference MCY/99/31. The results of a study to determine accuracy and precision, LODs and stability of HCC in sheep tissues were provided.

Accuracy and precision: For sheep liver and kidney, the sponsor noted that three fortification concentrations corresponding to MRL (20µg/kg), ½x MRL and 2x MRL were used for recovery determinations on day 1 (n=6 at each fortification level) in the original method. Three more fortified tissue samples were analysed, at each of the above concentration levels, on Day 2 and Day 3 and involving more than one operator in order to determine the within laboratory reproducibility part of precision. For sheep fat, the accuracy and precision were determined using the same procedure employed for liver and kidney, but fortification concentrations levels corresponded to the fat revised MRL (200µg/kg), 1/2x MRL and 2x the MRL. For muscle, the accuracy and precision were determined in a similar way but using the sheep fat extraction procedure to overcome the chromatographic problems due to the variable fat content of muscle. Spiking levels were the same as for liver and kidney (identical MRL). For fat, recoveries and coefficient of variation (CV) at each respective level (100, 200 and 400 µg/kg) were: 95.0% (8.9), 83.9 % (10.1), 90.4% (5.1), respectively. Interday variation of fat analysis had a mean CV of 11.0% (n=12). For liver, recoveries and coefficient of variation (CV) at each respective level (10, 20 and 40 µg/kg) were: 94.2% (5.6), 85.2% (6.3), 90.0% (8.0), respectively. Interday variation of liver analysis had a mean CV of 12.9% (n=12). Similarly, for kidney recoveries at the same levels were 81.6% (9.0), 95.2% (5.9) and 85.8% (8.7), respectively. Interday variation of kidney analysis had a mean CV of 13.4 % (n=12). For muscle, recoveries at the same levels were 85.3% (11.6), 90.5% (5.1) and 74.6% (7.5), respectively. Interday variation of muscle analysis had a mean CV of 11.3% (n=12).

Limit of detection (LOD): This was calculated as the mean background level at the retention time of HCC plus 3x SD of the mean from the analysis of 20-21 independently extracted tissue blank samples. Samples were processed as per original method MCY/99/31 for the liver, kidney and fat but using the fat extraction technique for muscle. From the calculations, an LOD of 5µg/kg was proposed for all tissues.

Stability: The stability of HCC in the final tissue hexane extracts was assessed by spiking tissues with a particular level and processing them as per original method MCY/99/31 as regards the liver, kidney and fat but using the fat extraction technique for muscle. The extract were kept under ambient conditions and analysed on three occasions. The results indicated that a stability period of 4-5 days for each tissue type was acceptable. The sponsor provided raw data presented as the following chromatograms: a typical sheep tissue blank, a typical HCC standard for each tissue spiked at the MRL level (20µg/l), fat and muscle spiked with HCC at the LOQ level.

APPRAISAL

Alpha-cypermethrin is a pyrethroid insecticide consisting of two of the four cis isomers present in cypermethrin (100% cis-isomers). These isomers comprise the most biologically- active enantiomeric pair. It is used in veterinary medicine. Cypermethrin consists of a mixture of 4 cis- and 4 trans-isomers (contains 20-40% alpha-cypermethrin). It may be used as a pesticide or as a veterinary drug in at least two formulations: either 45:55 cis:trans or 80:20 cis:trans, named high cis cypermethrin (HCC).

Studies on metabolism and residues of both compounds (FNP 41/9, 41/13, 41/14) indicates that there is no interconversion of cis to trans isomers and that the trans isomers deplete more rapidly from treated animals than cis isomers. Consequently, the residues found after veterinary treatment with cypermethrin and alpha-cypermethrin consist only of cis isomers and the source of the residue might be difficult to determine.

Under standard analysis conditions, the isomers of cypermethrin were not resolved and a single fused peak was obtained.

The 58th JECFA noted to national authorities the possible difficulty to determine whether residue concentrations comply with the recommended MRLs since the MRLs for cypermethrin and alpha-cypermethrin are different.

No new depletion studies were presented to the 62th meeting of the Committee. Results of studies provided to the 58th meeting of the Committee, indicate that for alpha-cypermethrin residues in cattle treated at a 3mg/kg dose using a 14C-alpha-cypermethrin formulation, the maximum concentration of residues as analyzed by either by HPLC analysis with a radio-label detector or by GC-ECD were 647µg/kg for back fat, 421µg/kg for omental fat, 22µg/kg for kidney, 35 µg/kg for muscle and <30µg/kg for liver. For alpha-cypermethrin in sheep treated with a topical dose of 15 mg/kg, maximum concentration of residues were 1323µg/kg for back fat, 314µg/kg for omental fat, 22µg/kg for kidney and <20µg/kg for muscle and liver. In milk, the highest concentration of residues found were 89µg/kg (60h). For cypermethrin, in a study on sheep treated with the recommended topical dose, the highest concentration of residues found in fat measured using a GC-ECD method was 34µg/kg while residues in liver, muscle and kidney were below the LOQ (10µg/kg).

Three analytical GC-ECD methods for the determination of cypermethrin residues in cattle tissues (MCY/01/51), in sheep tissues (MCY/99/31) and in cattle milk (MC/01/50) were submitted to the present Committee. They were properly validated. The submission also enclosed supplements to the method reference MCY/99/31 for determining cypermethrin in sheep tissues (the stability of HCC in sheep kidney and LOD determination in sheep liver and muscle).

The methods describe the determination of the concentration of HCC present in cattle and sheep tissues and milk using gas chromatography and electron capture detection, following extraction. The methods have almost identical extraction procedure differing in two points: the solvents used in the first extraction (acetonitrile is used with preference to chlroroform for safety reasons) and different cartridges in solid phase extraction. The instrumental GC-ECD conditions were identical. The methods have been validated in a similar way. The following criteria were evaluated: linearity, accuracy and precision, assay specificity, stability and practicability and were found to be adequate. Methods are suitable for determining the concentration of HCC in cattle tissues and milk over the range of 10µg/kg to 400µg/kg.

The limit of detection (LOD) and limit of quantitation (LOQ) were estimated for all methods. For cattle and sheep tissues LOQs were 100µg/kg for fat and 10µg/kg for liver, muscle and kidney respectively. For cattle tissues LODs were 5µg/kg for fat and 2µg/kg for liver, muscle and kidney. For sheep tissues, LODs were 5µg/kg for all tissues. For cattle milk: LOQ was 10µg/kg and LOD was 6µg/kg.

RECOMMENDED MAXIMUM RESIDUE LIMITS

The following factors can be considered in recommending a suitable marker residue and one set of maximum residue limits for the entire cypermethirn group:

- Alpha-cypermethrin (100% cis) consists of two of the four cis isomers presented in cypermethrin.
- A common ADI of 0-20µg mg/kg body weight, equivalent to 0-1200µg/kg was established for the most toxicologically active substance by the present Committee.
- The metabolism of cypermethrin and alpha-cypermethrin is similar in all species studied.
- The parent drugs cypermethrin and alpha-cypermethrin were the only recommended marker residues by the previous Committees.
- Residues of cypermethrin and alpha-cypermethrin found after treatment consists only of cis isomers.
- Using the common analytical methods for residue control, the eight isomers of cypermethrin are not resolved and a single fused chromatographic peak is obtained. Therefore, residues are reported as the sum of all isomers.
- MRLs of alpha-cypermethrin in cattle and sheep tissues and cattle milk recommended by the 58th Committee were: muscle, liver and kidney 100µg/kg; fat 1000µg/kg and cattle milk 100µg/kg. MRLs in liver and kidney were recommended on the basis of the limit of quantification of methods (LOD=20 µg/kg for sheep tissues, 50µg/kg for cattle tissues). MRLs in fat, muscle and cattle milk were based on residue data of studies submitted for evaluation.

- MRLs of cypermethrin in sheep tissues recommended by the 58th Committee were 20µg/kg muscle, liver and kidney and 200µg/kg in fat. The MRL in muscle, liver and kidney were recommended using the limit of quantitation of the method (10µg/kg) as residues at 7 days post-treatment are above the LOQ only in fat tissue. MRLs for fat were based on the residue studies using a pour-on formulation reported at the 54th Committee.
- New submitted methods are suitable to determine residues of both substances as the sum of isomers with the following LOQs: LOQ=100µg/kg for fat, 10µg/kg for liver, muscle and kidney respectively for cattle and sheep tissues. For cattle milk: LOQ=10µg/kg.

In considering a common set of recommendations for residues of cypermethrin and alpha-cypermethrin in cattle and sheep tissues and rounding, as appropriate, the following MRLs, expressed as of total cypermethrin residues, are 50µg/kg for muscle, liver and kidney; 1000µg/kg for fat, and 100µg/kg for milk.

The recommended MRLs in muscle, liver and kidney are based on the limits of quantitation of the new methods considering that residues are ≤ 35 µg/kg in both cattle and sheep tissues.

The recommended MRLs for fat and cattle milk are based on residue data from studies using the recommended treatments.

Residues in sheep tissues are lower than in cattle tissues, therefore, the same MRLs can apply to both species.

Using the daily food consumption figures for the theoretical diet, the residue equivalents of cypermethrin and alpha-cypermethrin are summarized in Table 10 (368 µg). The pesticide exposure for cypermethrin calculated by JMPR is 300 µg, therefore, total theoretical exposure for the cypermethrins would be approximately 650 µg.

The previously recommended MRLs for cypermethrin and alpha-cypermethrin are replaced by the following MRLs in cattle and sheep, as equivalents of total cypermethrin residues, 50µg/kg for muscle, liver and kidney; 1000µg/kg for fat, and 100µg/kg for cattle milk.

These MRLs are recommended for consideration by JMPR to harmonize MRLs of cypermethrin and alpha-cypermethrin.

Table 10. Theoretical maximum daily intake of residues of cypermethrin

Tissue	Recommended MRL (µg/kg)	Food Consumption Factor (kg)	Ratio MR/TR	Cypermethrins Equivalents (µg)
Muscle	50	0.3	0.3	50
Liver	50	0.1	0.1	50
Kidney	50	0.05	0.05	50
Fat	1000	0.05	0.8	60
Cattle Milk	100	1.5	0.95	158
Total				**368 µg**

REFERENCES

Beyerbach, A.(2000) ^{14}C- cypermethrin: Absorption, distribution, metabolism and excretion in sheep.

Covance Laboratories, (1976). Report No. 1412/021-D1141, Sponsor submitted. Casilda J.E., Ueda, K., Gaughan, L.,C., Jao, L.T. and Sutherland, D.M. Structure-biodegradability relationships in pyrethroid insecticides, Arch. Environ. Contam. Tox., 3, 491-500.

Cameron, D.A., Redgrave, V.A., MacDonald, I.A. and Gillis, N.A. (1993). Alpha-cypermethrin: concentrations of residues in cattle tissues. HRC report SLL 279/931913.

Crovect (pour-on residue study in sheep). (2001). Inveresk Research Report 20933., Sponsor submitted

Crawford, M., Hutson, D.H. (1977a). The metabolic fate of WL43467 (cypermethrin). Metabolism and elimination of ^{14}C-aryl-labelled cis and trans isomers in rats. .TLGR 0131.77

Crawford, M., Hutson, D.H. (1977b). TLRG.0098.77. The elimination and retention of WL 43467 when administered dermally or orally to sheep.

Crawford, M., Hutson, D.H. (1977c). The metabolic fate of WL43467 (cypermethrin) in mammals. The fate of a single oral dose of ^{14}C-cyclopropyl WL 43467in the rat. Group Research Report. TLGR 0004.77

Crawford, M., Hutson, D.H. (1977d). The metabolic fate of WL43467 (cypermethrin). Metabolism and elimination of ^{14}C-aryl-labelled cis and trans isomers in rats. TLGR 0131.77

Crawford, M., Hutson, D.H. (1978). TLRG.0078.78. The elimination of residues from the fat of rats following the oral administration of [^{14}C-benzyl] WL 43481 (cis-WL 43467)

Doran, A.M., Mayer I.H., Khunachak, A. (1999). RENEGADE alpha-cypermethrin (CL900049): Validation of Analytical Methods SAMS 461-1 and SAMS 456-1 for the determination of alpha-cypermethrin residues in cattle tissues (muscle, fat, kidney and liver). Inveresk Protocol Number 366292. Cyanamid Protocol Number AP98PT01, sponsor submitted.

Doran, A.M., Mayer I.H., Khunachak, A. (2000). RENEGADE alpha-cypermethrin (CL 900049): Validation of Analytical Method HE355/20-01R for the determination of alpha-cypermethrin residues in sheep tissues (muscle, fat, kidney and liver). Inveresk Protocol Number 367343. Cyanamid Protocol Number AP99PT01, sponsor submitted

EMEA (1998a). European Agency for the Evaluation of Medicinal Products-Committee for Veterinary Medicinal Products. Alpha-cypermethrin, Summary Report. EMEA/MRL/402/98.

EMEA (1998b) European Agency for the Evaluation of Medicinal Products-Committee for Veterinary Medicinal Products. Cypermethrin, Summary Report. EMEA/MRL/403/98

EMEA (1998c). European Agency for the Evaluation of Medicinal Products-Committee for Veterinary Medicinal Products. Cypermethrin, Summary Report. EME/MRL/403/98

EMEA (2001a). European Agency for the Evaluation of Medicinal Products-Committee for Veterinary Medicinal Products. Alpha-cypermethrin, Summary Report. EMEA/MRL/800/01

EMEA (2001b). European Agency for the Evaluation of Medicinal Products-Committee for Veterinary Medicinal Products. Cypermethrin, Summary Report. EMEA/MRL/801/01

FAO (1996). Alpha-cypermethrin. Residues of some veterinary drugs in animals and foods, Report of the Forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, Rome, 4-13 June 1996. FAO Food and Nutrition Paper 41, 59-70.

FAO (1996). Cypermethrin. Residues of some veterinary drugs in animals and foods, Report of the Forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, Rome, 4-13 June 1996. FAO Food and Nutrition Paper 41, 40-58.

FAO (2000). Cypermethrin. Residues of some veterinary drugs in animals and foods, Report of the Forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, Rome, 2000. FAO Food and Nutrition Paper 41/13, 19-20

Francis, W.P. and Gill, J.P. (1989). Fluflenoxuron/Alpha-cypermethrin residues in sheep tissues following treatment with Pampass/Renegade mixtures by dip or pour-on.

HE355/20-01R (1998). Determination of alpha-cypermethrin and Flumethrin residues in sheep fat.

Morrison, B.J. and Richardson, K.A. (1994). WL 85871 (ACYP, FASTAC). The metabolism of ^{14}C WL85871 after repeated oral dosing in the lactating cow in-life phase metabolite profiling.

Phillips, M., McLean C.L., McLellan G.L. (2000). RENEGADE alpha-cypermethrin (CL 900049): The depletion rate and metabolic fate of [^{14}C] CL 900049 in the steer and lactating cow. Inveresk Research Study Protocol Number 162556. American Cyanamid Study No. M98A049GB1, sponsor submitted.

Phillips, M., McLean, C.L. (2000). RENEGADE alpha-cypermethrin (CL 900049): The depletion rate and metabolic fate of [^{14}C] CL 900049 in sheep. Inveresk Research Study Protocol Number 164710. Fort Dodge Animal Health, Sponsor submitted

Redgrave, V.A., Cameron, D.M., Elsom, L.F., Girkin, R. and Cheng, C. (1992). ^{14}C alpha-cypermethrin: concentration of residues in cattle milk and tissues. HRC report SLL 240/920997.

SAMS 456-1 (1988). Determination of residues of alpha-cypermethrin in milk. Gas liquid chromatographic method.

SAMS 461-1 (1988). Determination of residues of alpha-cypermethrin in animal tissues. Gas liquid chromatographic method

Sherren, A.J. (1988a). Residues of alpha-cypermethrin in milk following topical treatment of cows with "Renegade" pour-on in the UK.

Sherren, A.J. (1988b). Residues of alpha-cypermethrin in milk following topical treatment of calves with "Renegade" pour-on in the UK.

White, D.A. (1987). Report on residues of cypermethrin and alpha-cypermethrin in sheep tissues.

DORAMECTIN

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ADDENDUM

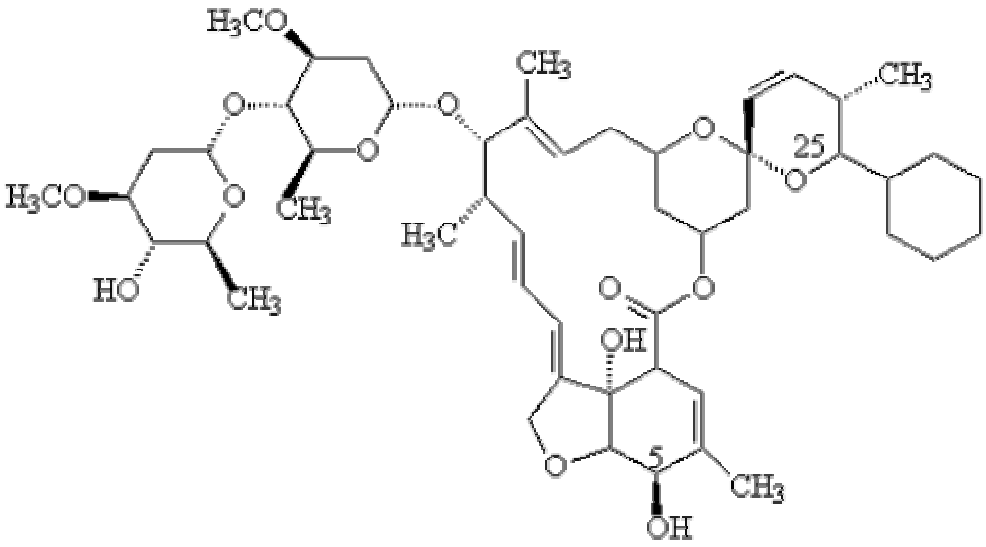
to the doramectin residue monograph prepared by the 45th meeting of the Committee in 1995, published in FAO Food and Nutrition Paper 41/8, and the 52nd meeting of the Committee in 1999, published in FAO Food and Nutrition Paper 41/12

IDENTITY

Chemical Name; 25-cyclohexyl-5-O-demethyl-25-de(1-methylpropyl)avermectin A_{1a}

Synonyms: Doramectin; Dectomax; UK-67,994

Structural formula:



Molecular formula: $C_{50}H_{74}O_{14}$

Molecular weight: 899.14

INTRODUCTION

Doramectin is an ecto- and endoparasiticide for use in cattle and pigs. It is a semi synthetic member of the avermectin class, structurally similar to abamectin and ivermectin. Previous evaluations by the Committee did not consider use in lactating cattle. The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Foods in 2002 requested the Committee to evaluate its use in lactating dairy cows.

Doramectin was first reviewed by the 45th Committee that established an ADI of 0-0.5 µg/kg bw on the basis of a NOEL of 0.1 mg/kg per day for mydriasis in a 3-month study in dogs treated with doramectin by gavage, using a safety factor of 200. The additional safety factor of 2 was applied because doramectin was not tested in CF-1 mice, the animal test considered at the time as the most sensitive to the neurotoxic effect of this class of drugs.. The Committee at its fiftieth meeting accepted the conclusions of the 1997 Joint FAO/WHO Meeting on Pesticide Residues (JMPR) that determined it was no longer necessary to apply an additional safety factor of 2 for the avermectins and milbemycins that had not been tested in CF-1 mice. On the basis of the decision at the fiftieth meeting of the Committee, the 58th Committee established an ADI of 0-1.0µg/kg bw.

The 58th Committee recommended the following MRLs for cattle: 10 µg/kg for muscle, 100 µg/kg for liver, 30 µg/kg for kidney and 150 µg/kg for fat, expressed as parent drug. Based on these values for the MRLs, the maximum theoretical intake would be 33 µg per day for a 60 kg person using the JECFA model diet.

In the dossier provided by the sponsor to the present Committee, information was presented for use of doramectin pour-on and injectable solution in lactating cattle in three new residue depletion studies. In addition, method performance data were provided for the analytical method to determine residues of doramectin in milk from lactating dairy cattle.

MILK RESIDUE STUDIES

Doramectin pour-on

These studies were designed to determine the profile of depletion of doramectin residues in milk following the administration of a 5 mg/ml doramectin pour on formulation when administered according to maximum labeled treatment regime to lactating dairy cattle

The first study using a pour-on treatment was conducted with twelve dairy Holstein cows. One animal with a medium level of production was randomly selected as a negative control, while the remaining eleven cows (one retained as a replacement animal) were treated with the test formulation. The cattle used in this trial were 3.5 to 9.5 years old weighing from 345 to 618 kg (mean 480 kg) at the time of treatment. The mean yield of milk was 13.28 liters and the mean milk fat was 4.91%

The treatment of the eleven animals with doramectin pour on formulation by topical application was at a dose rate of 0.58 mg/kg (1.0 ml/8.6 kg) doramectin and re-treatment with the same dose at 56 days later. The treatment was in accordance with Veterinary Health Research Standard Operating Procedure.

The sample collection during the initial phase (day 0 to 49) was by triplicate individual milk samples (collected immediately prior to treatment from all trial cattle on day 0), at both the morning and evening milking on days 0 to 7 days post treatment and at the morning milking on days 10, 16, 22, 28, 36 and 49 post treatment and at the evening milking on days 13, 19, 25, 32, and 40 On days 1, 4 and 10 an additional 100 ml sample was collected, refrigerated for 24 hours, separated into skim milk and milk fat with the individual portions subsequently stored frozen. During the second treatment from days 56 to 66 using the same topical dose; triplicate individual milk samples were collected from all trial cattle. at both the morning and evening milking on days 56, 57, 58, 59, 60, 61, 62 and 63 post treatment and at the morning milking on day 66.

Results –Study 1

The doramectin milk residue and milk/fat residue analysis were determined using a High Performance Liquid Chromatography validated method and fluorescence detection (LOQ; 3.12 µg/kg. The doramectin concentrations in milk increased from non-detectable concentrations at pre-treatment to a maximum mean value of 22.1 µg/kg at 72 hours post-dose. Between 60 and 120 hours post-dose, mean concentration were between 19.5 µg/kg and 22.1 µg/kg. The highest individual value (37.0 µg/kg) was observed at 72 hours post-dose. Mean doramectin residues decreased to a concentration below the limit of quantitation on day 16 (384 hours post-dose). Results are presented in Table 1a.

After re-treatment on day 56 using the same treatment as on day 0, doramectin residues increased gradually to a maximum mean value of 12.3 µg/ kg at 48 hours post-dose. The residues were constant between 48 and 96 hr post-dose, ranging between 10.1µg/ kg and 12.3 µg/kg and then decreasing to <LOQ at 240 hr post-dose. Results from re-treatment are summarized in Table 1b.

The milk/fat analyses were conducted at 1, 4, and 10 days post-dosing. Mean doramectin residues in the milk fat at these time points were 170.9 µg/kg, 501.4 µg/kg and 114.1 µg/kg, respectively. The concentration increases observed in the milk fat were consistent with the increases in doramectin residues in whole milk. The doramectin ratios in milk fat versus milk were calculated by dividing the measured concentration of each at the corresponding sampling times. Mean ratios at 1, 4 and 10 days were 29.6, 32.2 and 24.7, respectively.

The second study using the same pour-on treatment trial was conducted with twelve lactating cows, mean weight of 523.2 kilograms (392 - 620 kg) and mean milk production of 27.8 liters per day (23.2 - 32.1 liters) were selected. One animal was randomly selected to remain untreated as a negative control group and the remaining eleven cows were treated with the test formulation and one of the eleven treated cows was allocated to be a replacement animal and was sampled according to the trial schedule, however, the samples from this animal were not analyzed as no replacement of the initial study animals were required. These animals were treated with doramectin by topical route) and the dose rate of 0.58 mg/kg (1.0 ml/8.6 kg of pour on formulation) and re-treatment with the same dose 56 days later. The sample collection during the initial phase (day 0 to 49) was made by triplicate individual milk samples collected from all trial cattle at both the morning and evening milking on days 0 to 7 days post treatment and at the morning milking on days 10, 16, 22, 28, 36 and 49 post treatment and at the evening milking on days 13, 19, 25, 32, and 40. During the second treatment from days 56 to 66, triplicate individual milk samples were collected from all trial cattle at both the morning and evening milking on days 56, 57, 58, 59, 60, 61, 62 and 63 post treatment and at the morning milking on day 66. On days 1, 4 and 10 following re-treatment, an additional 100 ml sample was collected, refrigerated for 24 hours, separated into skim milk and milk fat with the individual portions subsequently stored frozen.

Results-Study 2

doramectin concentrations in milk increased gradually from non-detectable at pre-treatment to a mean of 8.9µg/kg. Individual maximum values were observed at day 1 and day 5 with the highest replicate value (15.8 µg/kg) observed at 33 hours post-treatment. Between 21 and 129 hours post-treatment, the group means milk doramectin residue concentrations were fairly constant, ranging between 6.1 and 8.9 µg/kg. Subsequently, doramectin residues decreased to concentrations below the limit of quantitation in all animals by the evening milking on day 19

Following re-treatment on day 56 using the same topical dose, residues increased to a mean maximum value of 8.2±5.2 µg/kg. at 93 hours. Individual maximum values were observed between the evening milking on day 58 and day 61 with the highest replicate value (21.7µg/kg) observed at 57 hours post-treatment. As seen the first treatment, residues were fairly constant between 33 and 141 hours post- re-treatment, with group mean values between 6.2 and 8.2 µg/kg before decreasing to <LOQ at 237 hours post re-treatment. Compared to the first treatment, doramectin milk residues were similar following re-treatment. Results are summarized in Table 2a and 2b

Milk fat analysis were conducted on samples collected on days 1 (21 hours), 4 (93hours) and 10 (237 hours) post treatment respectively. Mean doramectin residues in the milk fat at these times points were 90.8 µg/kg, 142.0 µg/kg and 55.1µg/kg respectively. The concentration increases observed in the milk fat were consistent with the increases in doramectin residues in whole milk. The highest individual milk fat concentration observed was 233.2 µg/kg. at 93 hours post treatment. The doramectin ratios in milk fat versus whole milk were calculated by dividing the measured concentrated of each at the corresponding sampling times. The mean ratios at 21, 93 and 237 hours post treatment were 14.2, 20.9 and 14.1 The highest calculated ratio was 48.5 at day 4.

Doramectin injectable formulation

This study was designed to determine the residue depletion profile of doramectin in milk following the administration of a 10 mg/ml doramectin injectable formulation to lactating cattle. Trial animals were lactating Holstein-Friesian.

Twenty four lactating cows were selected from al larger herd of 450 Holstein milking cows. Trial animals were selected to give a range of production levels representative of those in the larger herd. Two animals with milk production levels approximating the group mean were randomly selected to remain untreated as a negative control (treated with 0.9 % sodium chloride by subcutaneous injection). This study was conducted utilizing internationally acceptable standard operating procedures.

Trial cattle were individually weighed (on days 0 and 56) using calibrated electronic stock scales and animals treated by subcutaneous injection (on days 0 and 56) with the test formulation Injectable product at mean dose rates of 0.234 and 0.233 mg/kg individual body weight, respectively. These dose rates were equivalent to a dose volume of 1 mL per 42.65 body weight and 1 mL per 42.84 body weight.

During the initial phase (days 0 to 49), triplicate individual milk samples (replicates 1, 2 and 3) were collected from all trial cattle, at both the morning and evening milking on days 0, 1, 2, 3, 4, 5, 6, and 7 post treatment; at the morning milking on days 10, 16, 22, 28, 36, and 49 post treatment and at the evening milking on days 13, 19, 25, 32 and 40. An additional milk sample was collected at the morning milking on days 1, 4 and 10, refrigerated for 24 hours, separated into milk fat and skim milk portions and then stored frozen. During the second phase (days 56 to 66) triplicate individual milk samples were collected from all trial cattle, at both the morning and evening milking on days 56, 57, 58, 59, 60, 61, 62 and 63 post treatment and at the morning milking on day 66. Replicate 1 and 2 samples were frozen following collection, while replicate 3 samples were refrigerated. Replicate 1 samples were subsequently frozen on dry ice and forwarded to the analytical laboratory for doramectin milk residue analysis, Refrigerated replicate 3 samples were forwarded to a herd testing laboratory for milk fat content analysis and replicate 2 samples were retained frozen as back up samples, for analysis if required. Following separation into milk fat and skim milk portions, samples were forwarded frozen on dry ice to the designated analytical laboratory for milk fat doramectin residue analysis

Table 1a. Concentration of doramectin in milk (µg/kg) after treatment with a topical dose of 0.58 mg/kg in Southern Australia

	Hours	Concentration of doramectin (µg/kg) / cow number												
Day Time	Post-Dose	284	2236	2322	2643	2812	2967	3176	3394	3467	3516	Max	Mean	Std dev
Day 0 am	0													
Day 0 pm	12	1.6	1.6	1.6	3.7	1.6	3.9	3.2	4.0	1.6	1.6	4.0	2.4	1.1
Day 1 am	24	6.0	5.0	1.6	6.1	6.3	11.7	8.7	3.9	5.5	3.6	11.7	5.8	2.8
Day 1 pm	36	10.6	12.5	13.8	9.8	13.2	17.3	22.7	16.1	12.8	8.9	22.7	13.8	4.1
Day 2 am	48	15.8	13.3	18.1	11.8	18.2	19.2	25.0	11.5	17.7	8.9	25.0	15.9	4.7
Day 2 pm	60	17.6	16.7	20.5	15.2	23.7	17.4	22.8	27.3	21.5	12.0	27.3	19.5	4.5
Day 3 am	72	19.8	18.8	18.1	21.1	18.6	20.3	37.0	27.6	25.3	13.9	37.0	22.1	6.5
Day 3 pm	84	18.2	16.9	21.5	19.4	24.0	18.3	30.7	22.4	26.6	12.8	30.7	21.1	5.2
Day 4 am	96	14.7	15.2	10.0	10.6	21.9	19.3	23.2	14.5	29.6	10.6	29.6	17.0	6.4
Day 4 pm	108	20.8	15.2	23.8	16.3	21.5	17.5	34.4	24.9	24.1	9.2	34.4	20.8	6.8
Day 5 am	120	23.5	13.8	21.5	13.3	25.8	13.4	31.4	19.4	20.6	11.0	31.4	19.4	6.5
Day 5 pm	132	15.3	14.2	12.5	15.3	19.0	10.3	22.5	18.3	19.5	5.1	22.5	15.2	5.1
Day 6 am	144	13.8	10.4	8.9	11.2	15.4	8.5	27.3	17.5	16.1	4.1	27.3	13.3	6.4
Day 6 pm	156	9.4	11.0	7.5	8.2	12.3	8.1	14.1	13.9	12.2	3.5	14.1	10.0	3.3
Day 7 am	168	10.2	13.5	8.4	8.4	13.3	7.6	21.7	14.2	11.9	1.6	21.7	11.1	5.3
Day 7 pm	180	8.9	9.6	9.2	6.4	9.9	6.2	25.1	11.3	9.0	1.6	25.1	9.7	6.1
Day 10 am	240	3.3	4.7	3.4	1.6	6.7	5.6	10.8	1.6	4.5	1.6	10.8	4.4	2.9
Day 13 am	324	1.6	6.8	1.6	1.6	5.0	3.3	5.9	1.6	1.6		6.8	3.2	2.1
Day 16 am	384	1.6	4.3	1.6	1.6	3.4	1.6	1.6	1.6	1.6		4.3	2.1	1.0
Day 19 pm	468	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	
Day 22 am	528	1.6	1.6		1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	
Day 25 pm	612		1.6			1.6								
Day 28 am	672		1.6											
Day 32 pm	780		1.6											
Day 36 am	864													
Day 40 pm	972						1.6							
Day 49 am	1176													

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 1b. Concentration of doramectin in milk(µg/kg) after retreatment with a topical dose of 0.58 mg/kg in Southern Australia

	Hours	Retreatment with doramectin at a dose rate of 0.581 mg/kg												
Day Time	Post-Dose	284	2236	2322	2643	2812	2967	3176	3394	3467	3516	Max	Mean	Std dev
Day 56 am	0													
Day 56 pm	12	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	0.0
Day 57 am	24	7.3	4.3	1.6	3.2	4.4	4.2	5.5	3.3	7.5	6.6	7.5	4.8	1.9
Day 57 pm	36	3.3	6.0	1.6	4.6	5.7	8.2	9.1	3.7	14.4	9.9	14.4	6.6	3.8
Day 58 am	48	16.6	8.3	7.9	6.2	11.6	14.6	20.8	8.8	18.0	9.8	20.8	12.3	4.9
Day 58 pm	60	10.4	8.4	11.9	6.1	7.8	9.6	14.4	10.5	14.3	7.2	14.4	10.1	2.8
Day 59 am	72	13.5	9.8	11.1	4.8	11.5	13.0	20.3	4.4	16.6	7.3	20.3	11.2	5.0
Day 59 pm	84	13.7	10.2	11.1	6.1	10.2	11.9	16.9	10.9	15.9	7.5	16.9	11.4	3.4
Day 60 am	96	13.1	10.5	11.2	5.6	8.8	14.9	14.8	8.0	15.6	6.2	15.6	10.9	3.7
Day 60 pm	108	10.0	8.9	5.2	5.3	5.6	12.1	14.5	4.9	13.7	5.7	14.5	8.6	3.8
Day 61 am	120	10.9	9.2	9.6	5.3	8.7	10.9	15.2	7.5	12.4	3.5	15.2	9.3	3.4
Day 61 pm	132	9.3	9.5	7.8	6.2	6.1	9.4	12.7	5.3	11.3	5.9	12.7	8.3	2.5
Day 62 am	144	8.3	12.2	8.1	4.0	7.9	10.4	10.7	5.3	11.1	3.3	12.2	8.1	3.1
Day 62 pm	156	9.0	7.5	8.1	3.4	8.2	5.9	9.6	7.7	8.7	5.3	9.6	7.3	1.9
Day 63 am	168	7.6	8.0	7.6	4.5	5.0	7.1	11.3	7.7	8.6	4.6	11.3	7.2	2.1
Day 63 pm	180	4.7	5.9	5.7	3.9	4.6	5.9	7.9	1.6	6.8	1.6	7.9	4.8	2.1
Day 66 am	240	1.6	5.6	1.6	1.6	3.8	1.6	4.0	1.6	4.3	1.6	5.6	2.7	1.6

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 2a. Concentration of doramectin in milk (µg/kg) after treatment with a topical dose of 0.58 mg/kg in Northern Australia

	Hours	Concentration of doramectin (µg/kg) / cow number												
Day Time	Post-Dose	7	56	59	75	1408	1413	1623	1717	1754	1807	Max	Mean	Std dev
Day 0 am	0													
Day 0 pm	9	1.6	4.9	1.6	1.6	1.6	1.6	1.6		1.6	1.6	4.9	1.9	1.1
Day 1 am	21	4.8	14.1	6.4	10.0	1.6	4.3	13.8	6.1	1.6	1.6	14.1	6.4	4.8
Day 1 pm	33	6.7	15.8	6.7	7.1	1.6	8.3	13.1	3.7	4.2	4.8	15.8	7.2	4.3
Day 2 am	45	6.3	15.6	11.5	7.7	1.6	12.2	13.0	8.4	4.7	7.8	15.6	8.9	4.2
Day 2 pm	57	6.0	13.1	8.9	8.6	1.6	12.7	11.0	5.8	4.7	8.8	13.1	8.1	3.6
Day 3 am	69	5.6	11.3	7.9	4.8	1.6	11.2	10.0	6.0	4.2	9.2	11.3	7.2	3.3
Day 3 pm	81	5.0	6.2	10.0	4.7	1.6	7.5	9.4	3.6	4.1	9.0	10.0	6.1	2.8
Day 4 am	93	4.7	10.6	9.9	4.8	4.7	9.1	8.4	6.4	3.9	8.3	10.6	7.1	2.5
day 4 pm	105	4.9	12.9	9.4	7.8	5.9	10.4	7.3	5.9	4.4	7.9	12.9	7.7	2.6
Day 5 am	117	5.3	8.6	7.0	6.1	5.8	7.7	5.9	6.1	3.9	6.5	8.6	6.3	1.3
Day 5 pm	129	5.8	7.9	5.9	6.9	4.6	7.3	9.5	5.2	7.7	6.5	9.5	6.7	1.4
Day 6 am	142	4.1	5.9	5.4	4.5	4.7	6.7	5.6	4.6	5.8	5.4	6.7	5.3	0.8
Day 6 pm	153	3.7	5.1	5.3	3.4	4.8	5.2	6.4	4.2	4.8	4.7	6.4	4.7	0.9
Day 7 am	165	1.6	6.0	4.0	1.6	4.4	5.0	5.4	5.1	4.5	4.7	6.0	4.2	1.5
Day 7 pm	177	1.6	3.7	1.6	3.5	4.0	3.7	6.8	4.1	4.7	5.4	6.8	3.9	1.6
Day 10 am	237	1.6	3.2	3.6	1.6	1.6	1.6	3.6	4.5	4.5	5.3	5.3	3.1	1.4
Day 13 am	320	1.6	1.6	1.6	1.6	1.6	1.6	1.6	3.6	1.6	3.6	3.6	2.0	0.8
Day 16 am	381	1.6	1.6	1.6	3.1	1.6	1.6	1.6	1.6	1.6	1.6	3.1	1.7	0.5
Day 19 pm	464	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6		1.6	
Day 22 am	525	1.6	1.6	1.6	1.6	1.6		1.6	1.6	1.6	1.6		1.6	
Day 25 pm	608	1.6	1.6	1.6	1.6	1.6		1.6	1.6	1.6	1.6		1.6	
Day 28 am	669		1.6	1.6	1.6	1.6		1.6	1.6	1.6	1.6		1.6	
Day 32 pm	777		1.6		1.6					1.6	1.6			
Day 36 am	862		1.6							1.6				
Day 40 pm	968					1.6				1.6				
Day 49 am	1173					1.6				1.6				

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 2b. Concentration of doramectin in milk (µg/kg) after retreatment with a topical dose of 0.58 mg/kg in Northern Australia

	Hours	Retreatment with doramectin at 0.581 mg/kg										Max	Mean	Std dev
Day Time	Post-Dose	7	56	59	75	1408	1413	1623	1717	1754	1807			
Day 56 am	0						1.6			1.6				
Day 56 pm	9	5.5	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	5.5	2.0	1.2
Day 57 am	21	9.9	3.8	1.6	5.8	1.6	1.6	1.6	3.4	4.4	1.6	9.9	3.5	2.7
Day 57 pm	33	11.1	5.5	3.4	13.8	1.6	1.6	6.0	12.1	5.3	1.6	13.8	6.2	4.6
Day 58 am	45	10.7	5.4	1.6	10.0	1.6	1.6	5.9	14.0	7.1	4.8	14.0	6.3	4.3
Day 58 pm	57	12.8	6.0	1.6	12.7	3.2	1.6	6.8	21.7	5.8	9.3	21.7	8.1	6.2
Day 59 am	69	7.2	5.0	4.0	6.8	1.6	1.6	5.8	18.1	5.8	6.7	18.1	6.3	4.6
Day 59 pm	81	5.7	6.0	5.0	11.7	4.4	3.9	4.9	15.9	5.1	8.8	15.9	7.1	3.9
Day 60 am	93	8.8	9.2	6.1	18.2	1.6	3.9	4.5	15.8	5.5	8.4	18.2	8.2	5.2
Day 60 pm	105	9.2	9.8	5.4	12.5	1.6	3.2	4.4	7.7	6.4	8.0	12.5	6.8	3.3
Day 61 am	117	7.4	8.7	7.6	13.9	1.6	3.7	4.4	16.6	7.1	8.1	16.6	7.9	4.5
Day 61 pm	129	8.0	9.1	5.5	10.7	1.6	1.6	4.5	10.6	6.4	6.9	10.7	6.5	3.3
Day 62 am	141	7.0	6.8	3.9	11.6	1.6	1.6	4.8	11.4	6.6	7.9	11.6	6.3	3.5
Day 62 pm	153	1.6	5.3	1.6	6.6	1.6	1.6	4.6	11.4	5.1	4.7	11.4	4.4	3.1
Day 63 am	165	7.8	5.2	5.7	7.8	1.6	3.7	4.7	10.9	5.0	6.0	10.9	5.8	2.6
Day 63 pm	177	6.5	4.4	4.9	5.9	1.6	1.6	3.5	6.1	4.5	3.9	6.5	4.3	1.7
Day 66 am	237	1.6	1.6	1.6	5.3	1.6	1.6	1.6	4.3	3.5	3.7	5.3	2.6	1.4

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3a. Concentration of doramectin in milk (µg/kg) after injectable treatment of 0.23 mg/kg in Northern Australia – Group 1

	Hours	Concentration of doramectin (µg/kg) / cow number													
Day Time	Post-Dose	29	497	551	552	563	575	646	656	673	685	445	Max	Mean	Std dev
Day 0 am	0														
Day 0 pm	7	7.4	3.7	4.0	9.4	6.2	9.4	8.9	8.5	7.1	15.4	12.2	15.4	8.4	3.4
Day 1 am	19	12.3	11.7	17.2	20.8	36.3	10.6	36.6	21.7	21.0	44.8	29.0	44.8	23.8	11.4
Day 1 pm	31	22.2	18.0	21.8	36.2	57.1	15.4	56.5	30.7	28.9	50.4	46.61	57.1	34.9	15.5
Day 2 am	43	28.1	18.7	23.4	42.2	56.7	16.4	57.7	37.9	30.1	67.5	51.0	67.5	39.1	17.3
Day 2 pm	55	31.5	24.5	33.3	1.6	71.4	16.1	60.8	33.7	41.1	76.3	54.39	76.3	40.4	23.2
Day 3 am	67	36.3	26.3	33.0	50.9	64.2	39.5	54.7	34.5	39.7	76.3	54.73	76.3	46.4	15.1
Day 3 pm	78	35.3	22.1	35.3	58.8	35.5	32.5	53.7	23.8	23.1	77.7	32.64	77.7	39.1	17.3
Day 4 am	91	37.0	26.6	50.2	46.5	77.4	41.5	47.2	43.8	46.4	75.9	53.42	77.4	49.6	15.1
day 4 pm	102	33.9	26.7	33.9	44.1	58.4	28.8	45.2	25.0	50.0	65.2	49.49	65.2	41.9	13.3
Day 5 am	116	33.4	26.6	46.1	43.3	52.2	43.1	36.4	42.0	41.7	60.5	48.73	60.5	43.1	9.2
Day 5 pm	127	32.7	36.9	46.2	37.1	52.4	45.9	37.2	32.4	40.7	56.2	43.5	56.2	41.9	7.7
Day 6 am	139	31.7	31.2	31.5	35.9	49.0	42.7	30.9	28.6	38.8	40.1	39.34	49.0	36.3	6.2
Day 6 pm	151	28.7	26.7	30.5	26.2	43.0	37.9	30.0	21.6	32.2	31.5	36.55	43.0	31.3	6.0
Day 7 am	163	24.0	22.7	26.2	25.7	40.8	46.1	25.4	18.0	29.4	23.0	39.52	46.1	29.2	8.9
Day 7 pm	175	27.2	25.5	30.8	22.7	36.0	31.6	21.3	16.7	30.4	27.4	31.11	36.0	27.3	5.5
Day 10 am	236	15.2	15.5	14.0	14.7	19.0	21.0	13.9	8.9	25.9	10.9	25.13	25.9	16.7	5.4
Day 13 pm	318	8.7	11.8	9.5	8.5	10.4	13.0	3.9	4.2	16.7	4.1	20.56	20.6	10.1	5.3
Day 16 am	380	4.1	6.7	4.5	4.5	5.6	7.4	1.6	1.6	11.1	1.6	11.24	11.2	5.4	3.5
Day 19 pm	462	3.2	7.9	3.5	1.6	3.3	3.9	1.6	1.6	7.2	1.6	10.06	10.1	4.1	3.0
Day 22 am	524	1.6	1.6	3.8	1.6	1.6	1.6	1.6	1.6	5.5	1.6	8.2	8.2	2.7	2.2
Day 25 pm	606	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	3.8	1.6	4.7	4.7	2.1	1.1
Day 28 am	668	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6		1.6		1.6	
Day 32 pm	780	1.6	1.6	1.6	1.6	1.6	1.6			1.6	1.6	1.6			
Day 36 am	860	1.6	1.6	1.6	1.6		1.6			1.6					
Day 40 pm	966						1.6								
Day 49 am	1172														
Day 56 am	1340														

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3b. Concentration of doramectin in milk (µg/kg) after a second injectable treatment of 0.23 mg/kg in Northern Australia - Group 1

	Hours	Retreatment Concentration of doramectin (µg/kg) / cow number											Max	Mean	Std dev
Day Time	Post-Dose	29	497	551	552	563	575	646	656	673	685	445			
Day 56 pm	7	7.0		6.6	8.6	7.8	9.8	9.8	10.7	6.6	23.4	3.7	23.4	9.7	5.6
Day 57 am	22	40.6		19.4	36.0	17.1	24.9	27.7	28.0	14.9	35.1	19.4	36.0	24.7	7.6
Day 57 pm	31	38.0		26.3	73.7	32.1	29.0	38.8	34.0	20.8	51.8	31.0	73.7	37.5	16.1
Day 58 am	45	30.4		23.7	46.2	26.9	29.0	44.8	32.5	18.2	42.8	29.1	46.2	32.6	9.9
Day 58 pm	56	51.0		33.5	134.7	81.3	26.9	51.9	38.4	20.3	61.6	29.3	134.7	53.1	36.2
Day 59 am	70	104.3		27.9	43.9	44.8	27.8	48.1	33.8	25.1	54.5	30.6	54.5	37.4	10.6
Day 59 pm	79	92.6		32.4	71.0	67.3	33.8	55.7	37.0	17.9	61.5	34.7	71.0	45.7	18.5
Day 60 am	93	53.7		25.4	43.1	41.8	32.3	46.5	28.9	21.3	40.9	33.5	46.5	34.9	8.7
Day 60 pm	104	51.6		24.6	54.2	43.0	28.8	50.8	28.0	28.8	41.9	32.2	54.2	36.9	10.8
Day 61 am	117	44.1		24.1	35.8	34.5	27.0	44.0	26.7	29.0	38.4	30.6	44.0	32.2	6.4
Day 61 pm	128	49.5		24.6	39.4	46.4	22.3	44.6	25.5	25.0	31.2	30.0	46.4	32.1	9.1
Day 62 am	141	38.3		26.2	35.4	30.3	20.0	37.0	25.2	28.5	28.1	31.7	37.0	29.1	5.2
Day 62 pm	151	42.9		28.8	39.0	36.5	21.6	39.8	14.6	29.2	22.6	31.3	39.8	29.3	8.5
Day 63 am	167	33.1		23.2	32.3	28.2	25.9	32.7	25.0	23.3	19.0	33.5	33.5	27.0	5.0
Day 63 pm	175	37.2		29.7	36.5	38.2	18.7	36.2	71.7	30.4	20.3	39.5	71.7	35.7	15.4
Day 66 am	237	31.6		23.1	24.4	27.6	23.3	26.0	40.6	29.3	12.0	24.8	40.6	25.7	7.4

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3c. Concentration of doramectin in milk (µg/kg) after injectable treatment with a dose of 0.23 mg/kg in northern Australia – Group 2

	Hours	Concentration of doramectin (µg/kg) / cow number										Max	Mean	Std dev
Day Time	Post-Dose	786	816	2546	2721	2774	7241	7244	8272	9329	9359			
Day 0 am	0													
Day 0 pm	7	10.4	12.9	6.7	11.1	26.0	5.4	8.2	14.5	9.7	5.6	26.0	11.0	6.1
Day 1 am	19	32.0	29.3	17.8	24.9	50.3	18.8	16.9	27.1	24.2	9.6	50.3	25.1	11.1
Day 1 pm	31	31.8	46.4	27.1	46.7	69.8	49.3	23.9	46.3	41.3	25.3	69.8	40.8	14.2
Day 2 am	43	14.5	43.1	34.2	46.2	63.9	47.6	32.5	53.6	49.6	23.4	63.9	40.9	14.8
Day 2 pm	55	45.4	41.6	41.2	46.4	80.0	37.8	24.4	41.5	51.6	26.7	80.0	43.7	15.3
Day 3 am	67	41.9	39.6	41.7	41.9	68.3	33.2	20.2	37.3	49.1	24.8	68.3	39.8	13.2
Day 3 pm	79	36.3	36.7	33.8	43.2	31.8	31.8	27.9	28.8	43.1	25.9	43.2	33.9	6.0
Day 4 am	91	38.8	34.1	53.7	43.1	51.8	27.9	30.4	37.0	45.1	31.4	53.7	39.3	8.9
Day 4 pm	103	32.5	30.9	36.8	44.5	29.9	27.8	22.3	40.1	38.1	22.8	44.5	32.6	7.3
Day 5 am	115	35.8	31.0	39.4	45.7	38.6	24.3	21.7	39.1	36.4	31.7	45.7	34.4	7.3
Day 5 pm	127	29.6	29.7	33.4	46.7	40.4	29.2	21.9	36.8	34.5	32.1	46.7	33.4	6.8
Day 6 am	139	26.7	24.7	28.1	42.8	29.7	32.1	22.5	29.0	31.2	32.1	42.8	29.9	5.5
Day 6 pm	151	19.5	21.4	33.4	24.6	23.6	27.3	23.3	29.9	28.6	29.8	33.4	26.1	4.4
Day 7 am	163	17.8	18.1	31.9	29.0	16.1	25.7	16.9	30.7	25.0	29.0	31.9	24.0	6.2
Day 7 pm	175	16.4	16.8	22.0	33.8	22.5	26.0	21.1	25.5	29.2	21.4	33.8	23.5	5.3
Day 10 am	235	9.7	8.6	16.2	24.4	12.1	19.7	13.4	15.1	12.1	21.7	24.4	15.3	5.2
Day 13 pm	319	4.1	4.3	6.0	18.2	6.8	11.4	10.1	14.6	4.5	15.6	18.2	9.5	5.2
Day 16 am	379	1.6	1.6	3.1	11.2	3.6	6.2	6.0	9.2	1.6	11.8	11.8	5.6	4.0
Day 19 pm	463	1.6	1.6	1.6	8.9	1.6	3.3	5.9	7.1	1.6	11.3	11.3	4.4	3.6
Day 22 am	523	1.6	1.6	1.6	5.2		1.6	1.6	1.6	1.6	8.7	8.7	2.8	2.5
Day 25 pm	607	1.6	1.6	1.6	3.5			1.6	1.6	1.6	5.3	5.3	2.3	1.4
Day 28 am	667	1.6	1.6	1.6	1.6				1.6	1.6	4.4	4.4	2.0	1.1
Day 32 pm	775		1.6	1.6	1.6				1.6	1.6	1.6		1.6	
Day 36 am	859			1.6						1.6	1.6		1.6	
Day 40 pm	967													
Day 49 am	1176													
Day 56 am	1340													

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Table 3d. Concentration of doramectin in milk (µg/kg) after a second treatment with a dose of 0.23 mg/kg in northern Australia – Group 2

	Hours	Retreatment: Concentration of doramectin (µg/kg) / cow number										Max	Mean	Stdev
Day Time	**Post-Dose**	**786**	**816**	**2546**	**2721**	**2774**	**7241**	**7244**	**8272**	**9239**	**9359**			
Day 56 am														
Day 56 pm	7	9.1	5.4	6.3	18.7	16.1	9.7	8.6	7.7	9.4	7.2	18.7	9.8	4.3
Day 57 am	22	27.7	19.1	13.2	47.1	57.9	24.3	13.1	17.1	23.3	17.8	57.9	26.0	14.9
Day 57 pm	31	35.0	24.5	27.2	51.3	82.8	28.7	22.6	26.0	32.3	26.4	82.8	35.7	18.5
Day 58 am	45	35.3	23.6	22.1	52.0	77.9	28.7	17.1	19.4	30.6	25.3	77.9	33.2	18.6
Day 58 pm	56	38.7	29.5	33.3	47.2	83.4	29.9	29.6	37.5	37.5	28.1	83.4	39.5	16.5
Day 59 am	70	42.8	26.7	28.5	50.7	79.9	30.5	17.4	33.2	33.6	23.0	79.9	36.6	17.9
Day 59 pm	79	35.1	43.6	33.1	52.4	78.3	32.9	30.1	37.6	37.7	26.9	78.3	40.8	15.0
Day 60 am	93	36.6	28.5	22.6	44.5	65.6	30.0	17.4	29.4	30.2	23.3	65.6	32.8	13.8
Day 60 pm	104	35.6	45.9	33.0	40.6	72.3	29.8	26.6	35.9	32.8	24.2	72.3	37.7	13.7
Day 61 am	117	32.9	24.4	23.0	41.5	57.3	27.2	24.7	28.8	29.4	20.6	57.3	31.0	11.0
Day 61 pm	128	28.8	37.3	33.5	34.5	48.1	26.5	23.4	30.6	28.8	21.5	48.1	31.3	7.7
Day 62 am	141	35.1	20.3	19.4	32.6	41.9	23.4	37.3	25.2	29.0	21.7	41.9	28.6	7.8
Day 62 pm	151	30.7	26.8	18.1	29.4	25.9	18.8	30.9	23.1	28.8	22.7	30.9	25.5	4.7
Day 63 am	167	29.5	17.7	15.5	27.0	38.2	23.8	22.0	25.5	26.0	20.7	38.2	24.6	6.4
Day 63 pm	175	28.3	21.9	19.1	28.5	57.3	27.3	26.4	37.3	29.0	23.4	57.3	29.8	10.8
Day 66 am	237	24.5	13.8	11.6	19.0	21.1	19.3	17.2	21.0	18.3	13.4	24.5	17.9	4.0

Note: * indicates <LOQ. For estimates of mean values of doramectin residues and standard deviations, one-half the LOQ was used (1.6µg/kg). Non detect values were considered as zero and not used in the calculations.

Results

To facilitate sample handling, storage and analysis, cattle samples from trial were split into two groups by the Analytical Laboratory. The doramectin milk residue analysis was done using the same High Performance Liquid Chromatography – fluorescence detection method noted previously.

Doramectin concentrations in milk from animals treated with the injectable formulation increased gradually from non detectable concentrations at pre-treatment to a maximum mean value of 44.7 µg/kg at the morning milking on day 4 (91 hours post-treatment). Maximum individual milk doramectin concentrations occurred between the evening milking on day 1 and the morning milking on day 7, with the highest individual replicate value observed (80.0 µg/kg) at the evening milking on day 2 (55 hours post treatment). Between the evening milking of days 1 and 5 (31 to 127 hours post treatment) mean milk doramectin concentrations were fairly constant, ranging between 36.7 µg/kg and 44.7 µg/kg. Subsequently, milk doramectin residues gradually declined, with residues below the limit of quantitation in 9 of the 21 treated cows by the evening milking on day 19 (462 hours post treatment) and to below the LOQ in all animals by the evening milking on day 32 (774 hours post-treatment).Results are summarized in Table 3a and 3c.

Following retreatment on day 56, doramectin residues increased to a maximum mean value of 46.2 µg/kg by the evening milking on day 58 (56 hours post-retreatment). Mean doramectin milk residues were fairly constant between 31 and 104 hours post-retreatment, with values from 32.7µg/kg to 46.2 µg/kg. Residues then decreased to a mean value of 22.1µg/kg by 10 days (237 hours) after re-treatment. Individual maximum milk doramectin concentrations occurred between the evening milking on day 58 and the evening milking on day 63, with the highest individual replicate values in the two data sets (134.7 and 83.4 µg/kg) observed at 56 hours post re-treatment. The overall milk residue depletion profiles following both the initial and second treatment were similar. Results for the retreatment in the two groups of lactating cattle are summarized in Tables 3b and 3d.

Milk fat analysis were conducted using samples collected at the morning milking on day 1, day 4 and day 10 post treatment. Overall mean doramectin residues in milk fat at these time points were 557.0µg/kg, 1036µg/kg and 353.8 µg/kg, respectively. The concentration increases observed in the milk fat were consistent with the increases in doramectin residues in whole milk. .

The amount of doramectin residues resulting from the treatment with the injectable formulated product is distinctly different – approximately three times higher. While there are several possible reasons for this, the implication for recommendations on MRLs is noteworthy.

METHOD VALIDATION STUDIES

This study was conducted to validate an analytical methodology suitable for the recovery and quantification of doramectin in bovine milk, and to determine the partitioning of doramectin between the aqueous and fat components of whole milk, following milk fat separation by standard techniques. One animal was chosen for the second objective; the animal utilized had medium milk production (24 liters per day). The animal was weighed and treated with a 5 mg/ml doramectin pour-on formulation at a dose rate of 1.0 ml/8.6 kg (0.581µg/kg). Samples were collected on day 0, with replicates 4 to 6 fortified with 25 µg/kg doramectin, Replicates 7 to 9 were fortified with 50 µg/kg doramectin, Replicates 10 to 12 were fortified with 100 µg/kg doramectin with the remaining replicates 13 to 20 retained frozen.

The analytical method is described - "*A Determinative Procedure for the Detection and Quantitation of Doramectin in Cattle Plasma and Milk*". In the method, aliquots of milk were fortified with doramectin (UK-67,994) and the internal standard (UK-71,674) where appropriate and extracted prior to analysis by the high performance liquid chromatography (HPLC)-fluorescence method previously referred to.

The detection and quantitation of doramectin residues is based on the extraction procedure from plasma and milk and requires subsequent conversion to a fluorescent derivative (trifluoroacetic anhydride, triethylamine and acetonitrile). The conversion of doramectin is carried out in the presence of a fixed quantity of internal standard similar in structure to doramectin. The HPLC conditions are as follows:

Mobile phase:50% acetonitrile:	30% tetrahydrofuran: 20% Milli Q water. The mobile phase was filtered through a 0.45 µm Millipore filter.
The conditions for analysis:	
Flow rate	1.2 mL/min
Injection volume	10-15 µL
Detection (fluorescence) -	470 nm; Excitation (fluorescence) - 360 nm
Chromatographic run time	7-18 min.

The chromatographic system was highly satisfactory in terms of column efficiency, peak resolution, peak symmetry (tailing factors <1.03), system precision and linearity of response was satisfactory.

The limit of detection (LOD) for doramectin residues in milk was 0.061 µg/kg, determined from the analysis of blank samples and using the mean value plus three standard deviations, with a limit of quantification (LOQ) set at 3.12 µg/kg, determined from the fortified concentration in the method studies where the mean accuracy of quantification was 94.8% with the mean percentage imprecision of 6.1%.

The linearity of the assay was determined in milk fortified with doramectin at 50, 100, 200 µg/kg and had good linearity; the mean quantification at these concentrations being 50.7, 101.3 and 199.6 µg/kg for the 50, 100 and 200 µg/kg fortified samples. For the 50 µg/kg samples, the intra-assay accuracy is 97.7 - 104.2% with an intra-assay imprecision range of 0.4 – 4.4% while the mean inter-assay (inter day) accuracy and imprecision is 101.5% ± 3.5%, respectively. For the 100 µg/kg fortified samples, the intra-assay ranged from 97.0 – 103.8% with an intra-assay imprecision range of 0.6 – 4.3%. The mean inter –assay (inter day) accuracy and imprecision is 101.3% ± 4.0% respectively. For the 200 µg/kg samples, the intra-assay accuracy ranged from 95.0 – 104.4% with an intra-assay imprecision range from 0.8 – 3.9%. The mean inter-assay (inter day) accuracy and imprecision is 99.8% ± 4.5%, respectively.

The intra-day accuracy and recovery for doramectin at the LOQ varies from 87.8 to 115.4% with an intra-day imprecision of 3.9% - 6.6%. The inter-day accuracy and imprecision at the LOQ is 102.4%±12.4%.

The intra-day accuracy/recovery for doramectin at 50 µg/kg ranged from 85.3 to 106.4% with an overall intra-day accuracy/imprecision of 95.9%±14.3%. The intra-day accuracy and recovery for doramectin at 100 µg/kg are from 96.8 - 102.3% with an overall accuracy/imprecision of 99.0±7.8%. The inter-day accuracy and imprecision at 200 µg/kg is from 102.3 - 105.2% with an overall accuracy and imprecision of 101.4±8.5%.

Doramectin in milk was stable following a three-time freeze-thaw cycle. The concentration of doramectin in milk was within 7% of the freshly prepared samples. Doramectin fortified control milk samples (fortified with doramectin at 25, 50 and 100 µg/kg) were stable under frozen storage conditions (-20°C) for up to 6 months. Concentrations at the end of the storage period differed <10% from the initial values. Report analysis of milk (incurred residues) collected after 1 and 4 days from the pre-experimental doramectin pour on treated cow indicated that doramectin ranged from -7.6% to +5.2% of the original quantitation after 3 months of frozen storage and +7.8% and +20.4% following 6 months of frozen storage. At concentrations above the LOQ, incurred doramectin milk residues were stable under frozen conditions for a period of at least 6 months.

The partition of doramectin into milk fat was determined by analysis of doramectin in butterfat prepared from the cream of milk. A slightly modified procedure to a determinative procedure for the quantification of doramectin in cattle fat was used for quantitation of doramectin residues in butterfat

The recovery of doramectin from butterfat at 50 µg/kg with a fixed quantity of UK-71,647 internal standard was 101.4% for doramectin and 108.9% for the internal standard. At a doramectin concentration of 100µg/kg and the same internal standard concentration the recoveries are 110.2% and 116.1%, respectively. The accuracy of estimation of doramectin in the 50 and 100 µg/kg butterfat samples is 103.3% and 105.9%, respectively, with a imprecision (%CV) of 5.7% and 6.5%, respectively. Results of all method performance are summarized in Table 4.

Table 4. Summary of Method Validation Parameters

Parameter	Results
Intra-day accuracy (imprecision): LOQ	87.8 - 115.4% (3.9 – 6.6%)
Intra-day accuracy (imprecision): 50 µg/kg	97.7 - 104.2% (0.4 – 4.4%)
Intra-day accuracy (imprecision): 100 µg/kg	97.0 - 103.8% (0.6 - 4.3%)
Intra-day accuracy (imprecision): 200 µg/kg	95.0 - 104.4% (0.8 - 3.9%)
Inter-day accuracy and imprecision: LOQ	102.4% ± 12.4%
Inter-day accuracy and imprecision : 50 µg/kg	101.5% ± 3.5%
Inter-day accuracy and imprecision: 100 µg/kg	101.3% ± 4.1%
Inter-day accuracy and imprecision 200 µg/kg	99.8% ± 4.5%
Intra-day recovery (doramectin): 50 µg/kg	85.3% - 106.4%
Intra-day recovery (doramectin): 100 µg/kg	96.8% - 102.3%
Intra-day recovery (doramectin): 200 µg/kg	102.3% - 105.2%
Inter-day recovery and imprecision: 50 µg/kg	95.9% ± 14.3%
Inter-day recovery and imprecision: 100 µg/kg	99.0% ± 7.8%
Inter-day recovery and imprecision: 200 µg/kg	101.4% ± 8.5%
Inter-day recovery (imprecision):	. 96.4% (. 18%)
Accuracy(imprecision) for DOR in butterfat (50 µg/kg)	103.3% ± 5.7%
Accuracy(imprecision) for DOR in butterfat (100 µg/kg)	105.9% ± 6.5%
Limit of Quantification of DOR in milk	3.125 µg/kg
Limit of Detection of DOR in milk	0.061 µg/kg
Linearity of response	r-squared >0.999
Doramectin Stability in Milk (3X Freeze-Thaw cycles)	Stable
Doramectin Stability in Milk (Extended Frozen Storage)	Stable for at least 6 months

APPRAISAL

The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) requested consideration of MRL for bovine milk. The sponsor submitted three new residue depletion studies for doramectin to extend its use to lactating cattle for the control of internal and external parasites. The recommended dosage for the pour-on formulation is 0.5 mg/kg bw and for the injectable formulation it is 0.2 mg/kg bw. At the present meeting two studies were reviewed using the pour-on formulation and one using the injectable formulation. In addition, performance data were provided for the analytical method to determine residues of doramectin in milk from lactating dairy cattle.

Milk Residue Studies.

The first study using a pour-on treatment was conducted with ten dairy Holstein cows. The treatment was at a dose of 0.58 mg/kg bw doramectin and re-treatment with the same dose 56 days later. Milk samples were collected for 49 days and 10 days, respectively, following the first and second treatments. The doramectin milk residue and milk/fat residue were determined using validated High Performance Liquid Chromatography and Fluorescence detector method. The doramectin residue concentrations in milk increased to a maximum mean value of 22µg/kg at 72 hours post-dose. Mean doramectin residues decreased to concentrations below the limit of quantitation (3µg/kg) at 384 hours (16 days). After re-treatment doramectin residues increased gradually to a maximum mean value of 12µg/kg at 48 hours post-dose; and decreased to less than 4µg/kg at 240 hr (10 days) post-dose. The milk/fat analyses were conducted at 1, 4, and 10 days post-dosing. Mean doramectin residues in the milk fat at these time points were 171µg/kg, 501µg/kg and 114µg/kg, respectively. The doramectin ratios in milk fat versus milk were estimated for each of the corresponding sampling times. Milk fat concentrations factors for doramectin residues were 29.6, 32.2 and 24.7, respectively.

In the second study, animals were treated with doramectin by a topical route (pour on) using a dose of 0.58 mg/kg and re-treatment with the same dose 56 days later. Milk samples were collected as the same study. Doramectin concentrations in milk increased to a maximum mean value of 9µg/kg at 45 hours post-dose and decreased to below the LOQ by 237 hours(10 days). Following re-treatment on day 56 residues increased to a mean maximum value of 8µg/kg at 93 hours and decreased to less than the LOQ at 237 hours (10 days) post re-treatment. Mean doramectin residues in the milk fat at 1, 4, and 10 days were 91µg/kg, 142µg/kg and 55µg/kg, respectively. Milk fat concentration factors for doramectin residues versus milk were 14.2, 20.9 and 14.1, respectively.

Differences in residue concentrations between the two studies were attributed to climatic and productions factors.

The third study determined the residue depletion profile of doramectin following the administration of subcutaneous 0.23 mg/kg doramectin injectable formulation in lactating cattle followed by re-treatment at the same dose 56 days later. Sampling followed the same protocol as the two previous studies. The doramectin milk residue analysis was conducted using the High Performance Liquid Chromatography-fluorescence detection method noted previously. Doramectin concentrations in milk increased gradually to a maximum mean value of 45µg/kg at 67 hours. Subsequently, doramectin residues gradually declined, with mean residues below LOQ at 523 hours (22 days). Following re-treatment, doramectin residues increased to a maximum mean value of 53µg/kg at 56 hours. Residues then decreased to a mean value of 25µg/kg at 237 hours(10 days) after re-treatment Residues resulting from the injection treatment were consistently higher at any given time point than from the pour-on formulation. Milk fat analysis were conducted using samples collected at the morning milking on day 1, day 4 and day 10 post treatment. Mean doramectin residues concentrations in milk fat at these time points were 557µg/kg, 1036µg/kg and 354µg/kg, respectively. Milk fat concentration factors were 24, 24.2 and 23.4 respectively.

Method Validation Studies

This study was conducted to validate analytical methodology for the recovery and quantitation of doramectin residues in bovine milk. In the method validation, aliquots of milk were fortified with doramectin and the internal standard and extracted prior to analysis by the high performance liquid chromatography (HPLC)-fluorescence method. The method is based on the extraction procedure used for tissue and requires on-column conversion to a fluorescence derivative. The limit of quantification (LOQ) was approximately 3µg/kg. The recovery estimated at the LOQ is 95%. Method performance data indicate it is suitable for use in residue depletion studies and for routine surveillance purposes.

MAXIMUM RESIDUE LIMITS

In considering MRLs for doramectin in milk, the Committee agreed to take into account the following factors:

- The acceptable daily intake for doramectin is 0-1µg/kg body weight, equivalent intake of up to 60 µg per day for a 60 kg person
- Based on MRLs for tissues in cattle and pigs, and the theoretical maximum daily intake of residues in tissue using 33µg/day, approximately 27 µg per day are available for milk.
- Based on its limited metabolism, the single component and the known large partitioning ratio for residues between milk fat and aqueous milk, the Committee considers that the ratio for marker residue to total residue for doramectin in milk would be equivalent to the ratio of doramectin residues in fat (0.80).
- The residue studies provided used a pour-on formulation at 0.58 mg/kg bw and the injectable formulation at 0.23 mg/kg, somewhat in excess of the recommended doses of 0.5 mg/kg bw and 0.2 mg/kg bw, respectively.

- The marker residue is doramectin.
- A suitable analytical method is available for determining residues in milk.

To accommodate the maximum daily intake of residues based in the ADI, The Committee recommends an MRL of 15µg/kg for doramectin residues in bovine milk with residues determined as parent drug.

Taking into account the doramectin marker residue to total residue ratio in milk (80%) and the MRL for residues for doramectin in milk, the theoretical daily intake from 1.5 kg of milk would be 28 µg per day. The estimated theoretical intake of doramectin residues from tissues and milk is 61µg.

The Committee draws attention to National regulatory authorities of the following comment:

The recommended MRL represents the highest value consistent with the residue limits permitted by the ADI. On the basis of the recommended 15 µg/kg MRL for doramectin in whole milk in cattle, it is important to note that this MRL may require milk discard times up to 240 hours for milking cattle based on one study using the pour-on treatment. Milk discard times would be approximately 480 hours following treatment using the injection formulated dose.

REFERENCES

Australian Pesticides & Veterinary Medicines Authority (2003). Evaluation Report for applicant proposal relevant to Doramectin pour-on endectocide. October 2003.

EMEA (1997). The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Summary report (2).

Pfizer Animal Health (2002a), "A field and laboratory study to determine the residue depletion profile of doramectin in milk following the administration of a 5 mg/ml doramectin pour on formulation in lactating dairy cattle" (site 1), Volume 3 ,October 2002. Study report 2539B-14-004 (PFD 1115).

Pfizer Animal Health (2002b) "A field and Laboratory study for the validation of the analytical procedures used in the quantitation of doramectin in milk from dairy cattle. Volume 2, October 2002. Study report 2939A-14-01-002 (PFD 1121).

Pfizer Animal Health (2003a), "Determination of the analytical depletion profile of doramectin in milk following the administration of a 5 mg/ml doramectin pour on formulation in lactating dairy cattle" (site 2), Volume 4, March 2002. Study report 2539B-14-01-004 (PFD 1253).

Pfizer Animal Health (2003b) "A field and Laboratory study to determine the residue depletion profile of doramectin in milk following administration of a 10 mg/ml doramectin injectable formulation in lactating dairy cattle. Volume 5, April 2003. Study report 2539A-14-01-003 (PFD 1205).

Pfizer Animal Health "monograph of doramectin MRL in bovine milk". Volume 1, August 2003.

LINCOMYCIN

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ADDENDUM

To the monograph and addendum prepared by the 54th and 58th meetings of the Committee and published in the FAO Food and Nutrition Papers 41/13 and 41/14

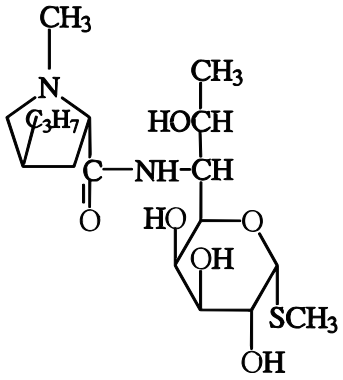
IDENTITY

Chemical Name: Methyl 6,8-dideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-D-erythro-∝-D-galacto-octopyranoside, monohydrochloride monohydrate (CAS name); CAS No. 154-21-2 (Lincomycin), 7179-49-9 (Lincomycin hydrochloride monohydrate); 859-18-7 (Lincomycin hydrochloride anhydrous)

Methyl 6,8-dideoxy-6-(1-methyl-trans-4-propoyl-L-2-pyrolidone-carboxamido)-1-thio-D-erythro-∝-D-galacto-octopyranoside monohydrochloride monohydrate, (IUPAC Name)

Synonyms: Lincomycin, Lincomycin hydrochloride, Upjohn: PNU-10149A, Albiotic® Non-Proprietary name: Lincocin

Structural formula:



Molecular formula: $C_{18}H_{34}N_2O_6S$

Molecular weight: 406.56

INTRODUCTION

Lincomycin is a member of the lincosamide antibiotics is produced by Streptomyces linconensis. It is used alone or in combination with other drugs in poultry and pigs for oral treatment of bacterial enteric infections, control of respiratory infections and growth enhancement. Intramuscular preparations are available for treatment of bacterial enteric and respiratory disease in calves. Combination preparations with neomycin are used as intramammary applications in lactating dairy cattle for treatment of acute mastitis.

Lincomycin was previously considered by the Committee at its fifty–fourth and fifty-eighth meetings. At its fifty-fourth meeting, the Committee established an ADI of 0 – 30 μg/kg body weight and recommended temporary MRLs for cattle, sheep and chicken tissues, and full MRLs for pig tissues. The temporary MRLs that were recommended for cattle tissues are: muscle 100 μg/kg, liver 500 μg/kg, kidney 1500 μg/kg, fat 100 μg/kg. The MRL recommended for milk was 150 μg/kg.

The Committee at the fifty-fourth meeting, also requested information on the following:

1. Data from residue depletion studies in cattle, sheep and chickens which show that lincomycin is the major microbiologically active residue in the edible tissues
2. Data from residue depletion studies showing that lincomycin is the major microbiologically active residue in chickens eggs
3. The results of a residue depletion study in which GC-MS is used to analyse residues in chickens eggs.

At the fifty-eighth meeting, data from new studies with broiler chickens and pigs were provided for evaluation and were used for reviewing the MRLS for chickens and pigs. Since lincomycin was determined in the new studies using three different analytical detection principles, namely, radioactivity, mass spectra and inhibition of microbial growth, it was concluded from the observed dose-linearity that parent lincomycin is the major microbiologically active residue in liver and kidney. From this approach, the Committee recommended full MRLs for chickens and pigs. However, the temporary MRLs for muscle, liver, kidney and fat for cattle and sheep recommended at the fifty-fourth meeting were withdrawn, as the requested information was not provided.

At the sixty-second meeting, the sponsor provided data from four cattle studies of which one was a new study (Barbiers and Smith, 1981), and three were studies that had been evaluated by the fifty fourth meeting (Weber et al, 1981; Hoffman et al, 1996, De Greave et al, 1997). One of the three previously evaluated studies was entirely on pharmacokinetics (Weber et al, 1981) and not tissue residues.

RESIDUES IN FOOD AND THEIR EVALUATION

Metabolism

Metabolic studies of lincomycin were evaluated during the fifty-fourth meeting. No data from cattle studies were available. Data from studies in pigs and chicken have shown that metabolism of lincomycin is rapid and lincomycin is the major component of the total residues.

Residue Depletion Studies with Unlabelled Drug

In the new study, 17 calves were given lincomycin by intramuscular administration at a dose of 5 mg per kg body weight twice on the first day of treatment followed by a single dose of 5 mg/kg body weight per day for four consecutive days (Barbiers and Smith, 1981). Groups of animals were killed at 1, 7, 14, 21 and 28 days after the last treatment. Samples of liver, kidney, muscle, fat and injection site were assayed for lincomycin residues using a microbiological method with a limit of detection (LOD) of 0.1 mg/kg. Results of the microbiological assay are shown in Table 1.

Table 1: Mean residue concentrations of lincomycin in tissues of calves given intramuscular injections of lincomycin (5 mg/kg body of weight) two times on the first day followed by one injection (5 mg/kg body of weight) daily for four consecutive days

Withdrawal time (days)	Mean residue concentrations (mg/kg)				
	Muscle	Liver	Kidney	Fat	Injection site
1	<LOD (5)	0.56 (5)	0.34 (5)	<LOD (3), * (2)	0.26 (5)
7	<LOD (3)	<LOD (3)	<LOD (3)	<LOD (3)	<LOD (3)
14	NA	NA	NA	NA	<LOD (3)
21	NA	NA	NA	NA	NA
28	NA	NA	NA	NA	NA

LOD: limit of detection (0.1 mg/kg - microbiological assay)
NA = samples were not analysed
* Zones did not resemble lincomycin
( ) Number of animals in a group

The second non-GLP study involved twenty veal calves allocated to four groups each of five animals (Hoffman et al, 1996). All the four groups were given lincomycin by intramuscular administration at a dose of 5 mg per kg body weight, the first two doses at 12 hours interval, followed by four doses at 24 hours interval. The animals were killed at 8 hours, 7, 14 and 21 days after the last dose and tissue samples taken and analysed for lincomycin using a validated GC/MS method with a limit of quantitation (LOQ) of 40-47 µg lincomycin free base equivalent /kg tissue. The results are summarised in Table 2.

Table 2: Mean residue concentrations of lincomycin in tissues of calves given intramuscular injections of lincomycin (5 mg/kg body of weight) and spectinomycin (10 mg/kg body of weight) two injections at an interval of 12 hours followed by four injections (5 mg/kg body of weight) at an interval of 24 hours.

Withdrawal time	Mean residue concentrations (mg/kg)				
	Muscle	Liver	Kidney	Fat	Injection site
8 hours	0.72	0.30	3.34	0.10	2.42
7 days	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14 days	<LOQ	0.07*	<LOQ	<LOQ	<LOQ
21 days	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

* One sample, the remaining 4 assayed were <LOQ
LOQ: limit of quantification (0.040 – 0.047 mg/kg - GC/MS)

In another study conducted according to GLP, sixteen cows were given three consecutive intramammary infusions of 330 mg of lincomycin into each of the four quarters of the udder at 12- hour intervals (De Grave et al, 1997). The animals were killed at 1, 7, 14 and 21 days after treatment and tissue samples taken and analysed by GC/MS, the results are summarised in Table 3.

Table 3: Mean residue concentrations of lincomycin in tissues of lactating cows given at 12-hour intervals three consecutive intramammary infusions containing lincomycin (300 mg) in each quarter

Withdrawal time (days)	Mean residue concentrations (mg/kg)			
	Muscle	Liver	Kidney	Fat
1	0.037	0.23	0.60	<LOQ
7	<LOQ	0.058	<LOQ	<LOQ
14	<LOQ	0.026	<LOQ	<LOQ
21	<LOQ	0.029	<LOQ	<LOQ

LOQ: limit of quantification (0.015 mg/kg - GC/MS)

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The various methods that have been used to determine the concentrations of lincomycin in foods of animal origin include microbiological assay, thin-layer chromatography-bio-autography, GC with alkaline flame detector and GC/MS. These were reviewed during the fifty-fourth meeting of the Committee. No new methods were submitted for review in the present meeting.

APPRAISAL

The Committee reviewed the data from the new study, and took into consideration the studies that were evaluated during the fifty-fourth meeting. These studies, taken together were considered insufficient to allow any extrapolation, such as the relationship between dose and extrapolated concentration at time zero after drug administration. Thus, the approach used for pig and chicken data during the fifty-eighth meeting of estimating parameters that fit a similar relationship irrespective of the method of residue analysis cannot be applied on the cattle data submitted. Establishment of dose-linearity relationship using data generated by radioactivity measurements, GC/MS and microbiological assay was sufficient to confirm that lincomycin is the major microbiologically active residue in edible tissues of pigs and chicken.

The sponsor has attempted to compare data from two calf studies (Barbiers and Smith, 1981; Hoffman et al, 1996) by estimating tissue residues in different tissues using plasma half-life obtained from a pharmacokinetic study of lincomycin in cows (Weber et al, 1981), but the Committee noted that data from non-ruminating calf studies could not be used to support data from studies on intramammary administration of the drug in cows. Residues of the drug were detected in liver for up to 21 days in cows, unlike in calves where the drug was detected in day one only.

In an attempt to establish MRLs, data from other species were also considered. This was however not possible since studies in pigs and chickens have shown significant differences between animal species in the kinetics of lincomycin residues in tissues. In pigs for example, concentrations of the drug in kidney were three times higher than those in liver, whereas in chickens, the concentrations were similar. At comparable doses, the concentrations of residues in muscle and skin/fat were also higher in pigs than in chickens. Therefore, the Committee concluded that it was not possible to extrapolate the kinetics of lincomycin residues between animal species.

MAXIMUM RESIDUE LIMITS

Since the available information was inadequate, the present Committee could not recommend MRLs for lincomycin in cattle tissues.

REFERENCES

Barbiers, A.R. and Smith, L.J. (1981) Lincomycin (U – 10, 149A) and Spectinomycin (U-18,409E) residues in tissues from non-ruminating calves following intramuscular injection of LINCO-SPECTIN* Sterile Solution. Upjohn Technical Report 772-9760-80-001. 19 January 1981.

De Grave, J., Van Heugen, I-C, Nappier, J.L. and Deluyker, R.A. (1997) Tissue residue depletion study of LINCO-SPECTIN Sterile following intramammary infusions to dairy cattle. Part II- lincomycin assay validation, and lincomycin residues. Pharmacia & Technical 804 – 7926 – 97 – 001, 11 February 1997.

Hoffman, G.A., Delahaut, P., De Graeve, J., Brown, S.A., Gilbertson, T.J., and Lens, S.T. (1996) Lincomycin residues in the tissues of calves at various times after multiple injections of LINCO-SPECTIN Sterile Solution at a dose rate of 15 mg per kg body weight (5 mg lincomycin + 10 mg spectinomycin/kg). Upjohn Technical Report X803-7926-95004, 29 January 1996.

JECFA (2000) Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper 41/13.

JECFA (2002) Residues of some veterinary drugs in animals and food. FAO Food and Nutrition Paper 41/13.

Weber, D.J., Barbiers, A.R., Lallinger, A.J. (1981) Pharmacokinetics of lincomycin in the bovine following intravenous and intramammary doses of Lincocin®. Upjohn Technical Report 7256-81-7256-001. 24 June 1981.