ISSN 0254-4725

FAO
FOOD AND
NUTRITION
PAPER

41/13

Residues of some veterinary drugs in animals and foods

Cyhalothrin
Cypermethrin
Dicyclanil
Flumequine
Ivermectin
Lincomycin
Melengestrol acetate
Permethrin
Trichlorfon

WORLD
HEALTH
ORGANIZATION

Food
and
Agriculture
Organization
of
the
United
Nations

Residues of some veterinary drugs in animals and foods

FAO
FOOD AND
NUTRITION
PAPER

41/13

Monographs prepared by the
fifty-fourth meeting of the
Joint FAO/WHO Expert Committee
on Food Additives

Geneva, 15-24 February 2000

WORLD
HEALTH
ORGANIZATION

Food
and
Agriculture
Organization
of
the
United
Nations

Rome, 2000

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

ISBN 92-5-104474-0

TABLE OF CONTENTS

54th JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES
Geneva 15-24 February 2000

Members

Professor A. Anadón, Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain

Dr L.-E. Appelgren, Professor of Pharmacology, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, The Swedish University of Agriculture Sciences, Uppsala, Sweden

Dr D. Arnold, Acting Director, Federal Institute for Health, Protection of Consumers and Veterinary Medicine, Berlin, Germany

Dr J. Boisseau, Director, Agence national du médicament vétérinaire, La Haute Marche, Javené, Fougères, France (*Vice chairman*)

Professor A.R. Boobis, Section of Clinical Pharmacology, Division of Medicine, Imperial College School of Medicine, London, England *(Joint Rapporteur)*

Dr L.D.B. Kinabo, Professor, Department of Veterinary Physiology, Biochemistry, Pharmacology and Toxicology, Sokoine University of Agriculture, Chuo Kikuu, Marogoro, Tanzania

Dr J.D. MacNeil, Head, Center for Veterinary Drug Residues, Health of Animals Laboratory, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada (*Joint Rapporteur)*

Dr J.G. McLean, Professor Emeritus, Swinburne University of Technology, Hawthorn, Victoria, Australia (*Chairman*)

Dr M.A. Miller, Food and Drug Administration, Rockville, MD, USA

Professor J. Palermo-Neto, Departamento de Patologia, Faculdade de Medicina Veterinaria, Universidade de São Paulo, São Paulo, Brazil

Dr J.L. Rojas Martinez, Chief, Toxicology and Residues Section, National Laboratory of Veterinary Sciences, Ministry of Agricultural and Animal Husbandry, San José, Costa Rica

Dr S. Soback, Head, National Residue Laboratory, Kimron Veterinary Institute, Ministry of Agriculture, Beit Dagan, Israel

Dr R.W. Stephany, Head, Laboratory for Residue Analysis, National Institute of Public Health and the Environment, Bilthoven, The Netherlands

Secretariat

Dr F. Aguilar, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland

Dr S.M.F. Calumpang, National Crop Protection Centre, University of the Philippines, Los Baños College, Laguna, Philippines *(FAO Consultant)*

Dr C. E. Cerniglia, Director, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, USA *(WHO Temporary Advisor)*

Dr. R. Ellis, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*acting FAO Joint Secretary*)

Dr L.G. Friedlander, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA *(FAO Consultant)*

Dr K. Greenlees, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA, *(WHO Temporary Advisor)*

Mr D.J. Hamilton, Principal Scientific Officer, Animal and Plant Health Service, Department of Primary Industries, Brisbane, Australia *(FAO Consultant)*

Dr R.J. Heitzman, Science Consultant, Newbury, Berkshire, UK *(FAO Consultant)*

Dr J.L. Herrman, Scientist, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)

Mrs E. Heseltine, Communication in Science, Lajarthe, Saint-Léon-sur-Vézère, France *(editor)*

Dr R.C. Livingston, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA *(FAO Consultant)*

Dr K. Mitsumori, Chief, Third Section, Division of Pathology, Biological Safety Research Centre, National Institute of Health Sciences, Tokyo, Japan *(WHO Temporary Advisor)*

Professor M.R.A. Morgan, Head, Procter Department of Food Science, University of Leeds, Leeds, UK *(FAO Consultant)*

Mr N Nakashima, International Programme on Chemical Safety, WHO, Geneva, Switzerland

Ir M.E.J. Pronk, Centre for Substances and Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, The Netherlands *(WHO Temporary Advisor)*

Mr D. Renshaw, Principal Scientist, Veterinary Products, Joint MAFF/Department of Health Food Standards and Safety Group, Department of Health, London, UK *(WHO Temporary Advisor)*

Dr L. Ritter, Executive Director, Canadian Network of Toxicology Centres, Professor, Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada *(WHO Temporary Advisor)*

Dr G. Roberts, Manager, Chemical Products Assessment Section, Therapeutic Goods Administration, Commonwealth Department of Health and Aged Card, Woden, ACT, Australia *(WHO Temporary Advisor)*

Dr B. Roestel, Head International Affairs, Agence national du médicament vétérinaire, La Haute Marche, Javené, Fougères, France *(FAO Consultant)*

Dr S. Sundlof, Director, Center for Veterinary Medicine, Food and Drug Administration, Rockville, MD, USA *(WHO Temporary Advisor)*

Dr A. Tejada, FAO Joint Secretary for JMPR, Pesticide Management Group, Plant Production and Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy

Professor F. R. Ungemach, Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany *(WHO Temporary Advisor)*

Dr R. Wells, Gordon, New South Wales, Australia *(FAO Consultant)*

Dr Y. Yamada, Food Standards Officer, Joint FAO/WHO Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy

Dr J. Zmudzki, Professor and Head, Department of Pharmacology and Toxicology, National Veterinary Research Institute, Pulawy, Poland *(FAO Consultant)*

ABBREVIATIONS USED IN THIS DOCUMENT

$[\alpha]_D$	-optical rotation
ADI	-Acceptable Daily Intake
AUC	-area under concentration-time curve
Av.	-average
b.i.d.	-twice a day
BP	-British Pharmacopoeia
Bp	-boiling point
Bq	-Becquerel (one disintegration/sec)
BW or bw	-body weight
°C	-degrees Celcius
^{14}C	-radiolabelled carbon-14
C_{max}	-maximum concentration
µCi	-microcurie (radioactivity)
cm^3	-cubic centimeter
conc	-concentration
CV	-coefficient of variation
D or d	-day
DPM or dpm	-disintegration per minute
ECD	-electron capture detector
e.g.	-for example
ELISA	-Enzyme linked immunosorbentassay
EP	-European Pharmacopia
Eq	-equivalents
F	-female
FDA	-U.S. Food and Drug Administration
FID	-flame ionisation detector
g	-gram
GC	-gas chromatography
GI	-gastrointestinal
GLC	-gas-liquid chromatography
GLP	-Good Laboratory Practice
GVP	-Good Veterinary Practice
H or h	-hour
^3H	-tritium (radiolabelled hydrogen)
HPLC	-high performance liquid chromatography
i.e.	-that is
I.M. or i.m	-intra muscular
i.m.i	-intra muscular injection
I.P. or i.p.	-intra peritoneal
I.V. or i.v.	-intra venous
K_{el}	-rate constant
Kg or kg	-kilogram (10^3 gram)
L or l	-litre
LC	-liquid chromatography
LOD	-limit of detection
LOQ	-limit of quantification
LSC	-liquid scintillation counting
M	-mole or molar
M	-male
Max or max	-maximum
µg	-microgram (10^{-6} gram)
µm	-micrometer (10^{-6} meter)
µl	-microlitre (10^{-6} litre)
mg	-milligram (10^{-3} gram)
min	-minute
ml	-millilitre (10^{-3} litre)
MR	-marker residue
MRL	-Maximum Residue Limit
MRT	-mean residence time
MS	-mass spectrometry
m/z	-mass ion fragment
No. or n	-number
NA or na	-not analysed, assayed or not available
ND or nd	-not detected
ng	-nanogram (10^{-9} gram)
NM or Nm	-not measured
nm	-nanometer (10^{-9} meter)
NMR	-nuclear magnetic resonance
NOEL	-no observed effect level
ppb	-parts per billion (1 in 10^9)
pK_a	-dissociation constant
ppm	-parts per million (1 in 10^6)
R or r	-regression coefficient
RIA	-radioimmunoassay
RSD	-relative standard deviation (interaboratory)
rsd	-relative standard deviation (intralaboratory
SA	-specific activity
s.c.	-subcutaneous
SD or σ	-standard deviation
SEM	-standard error of mean
Sic	-correctly spelled
s.i.d.	-once per day
$t_{½}$	-half life
T_{max} or t_{max}	-time for maximum
TLC or tlc	-thin layer chromatography
TMS	-trimethyl silyl
TR	-total residues
TRA	-total radio activity
UD	-unchanged drug
USDA	-U.S. Department of Agriculture
USP	-United States Pharmacopia
UV	-ultraviolet
V_D	-volume of distribution
V/v or v/v	-volume/volume measurement
WT	-withdrawal time
Wt	-weight
W/v or w/v	-weight/volume measurement
W/w or w/w	-weight/weight measurement
%	-percent
>	-greater than
<	-less than
≥	-equal or more than
≤	-equal or less than

INTRODUCTION

The monographs on the residues of, or statements on, the 9 compounds contained in this volume were prepared by the fifty-forth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 15-24 February 2000. JECFA 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], 50th14 and 52nd[15] meetings.

In response to a growing concern about mass-medication of food producing animals and the potential implications for human health and international trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984[16]. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA[17]. In response to these recommendations, the 32nd JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, the 34th, 36th, 38th, 40th, 42nd, 43rd, 45th, 47th, 48th, 50th and 52nd meetings of JECFA were also dedicated exclusively to evaluation of veterinary drugs.

The eleventh session of the CCRVDF, held in Washington, D.C., USA during September 1998, revised the priority list of veterinary drugs requiring evaluation. The twelfth session of the CCRVDF, held in Washington, D.C., USA met subsequent to the 54th meeting of the Committee and although providing a new priority list, those substances will have to be considered for a subsequent JECFA devoted exclusively to residues of veterinary drugs in food. The drugs evaluated during the 54th meeting of JECFA included these compounds from the eleventh session of CCRVDF except temephos and tilmicosin. The evaluation of these substances was postponed to a future meeting of the Expert Committee. Data regarding alpha-cypermethrin was received too late to be considered by the Committee and must be postponed to a future meeting.

The present volume contains monographs of the residue data on 9 of the 13 compounds on the agenda. Data sponsors withdrew two substances, temephos and tilmicosin, and a monograph for oxytetracycline was not prepared as no new data were provided. The data provider for alpha-cypermethrin did not complete and submit the new data in time to be considered by the fifty-fourth JECFA, however, it will be made available for a subsequent meeting. As no data were provided in response to the Committee's request the temporary MRLs for cypermethrin and alpha-cypermethrin were not retained by the Committee. Likewise as no data were provided regarding tilmicosin for sheep milk, that temporary MRL also was not retained. For one substance on the agenda, trichlorfon (metrifonate), the Committee proposed guidance MRLs as residues in tissues were not determined in any study conducted according to good practices in the use of veterinary drugs. With the agreements reached by an *ad hoc* meeting of a small group of experts from JECFA and JMPR, and in accord with one of its recommendations from the report of that meeting[18], participants from JMPR were invited to the fifty-fourth JECFA for purposes of harmonization with the Joint Meeting on Pesticide Residues and the Codex Committee on Pesticide Residues. Monographs prepared by these participants are included in this publication with those prepared by JECFA participants on the same substances.

Substances reviewed by the Committee for the first time included the antimicrobial agent, lincomycin, the four insecticides cyhalothrin, dicyclanil, permethrin and trichlorfon (metrifonate). One production aid, melengestrol acetate, had not been evaluated previously by the Committee. Of the nine substances evaluated at the fifty-forth meeting, the anthelminthic agent, ivermectin had been considered previously by the Committee. Similarly, one antimicrobial agent, flumequine, had been evaluated previously by the Committee. Regarding insecticides, cypermethrin and alpha-cypermethrin had been evaluated previously.

The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the present 54th meeting is included in Annex 1.

The assistance of the FAO experts and members of the Secretariat in preparing these monographs is gratefully acknowledged.

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, in press.

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

CYHALOTHRIN

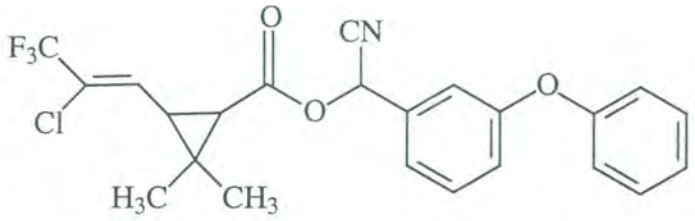
First draft prepared by
James MacNeil, Saskatchewan, Canada
Michael Morgan, Leeds, United Kingdom

IDENTITY

Chemical Name: (RS)-α-cyano-3-phenoxybenzyl (Z)-(1RS,3RS)-2-(chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate; CAS No. 68085-85-8

Synonyms: PP-563, Coopertix™, Grenade®

Structural formula:



Cyhalothrin is composed of two pairs of enantiomers, designated **A** and **B**, in an approximately 60:40 ratio (**A**:**B**);
A pair: *Z*(1*R*) *cis* (*R*) α-CN and *Z*(1*S*) *cis* (*S*) α-CN;
B pair: *Z*(1*R*) *cis* (*S*) α-CN and *Z*(1*S*) *cis* (*R*) α-CN.

Molecular formula: $C_{23}H_{19}ClF_3NO_3$

Molecular weight: 449.9

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Purity: The commercial product is a mixture consisting of 95% *cis*-isomers.

Appearance: Yellow to dark greenish-brown viscous liquid with no characteristic odour.

Melting point: Approximately 10°C

Boiling point: Decomposes >275 °C (atmos.); 187-190 °C (0.2 mm Hg)

Solubility: water, 0.003; acetone, >500; dichloromethane, >500; diethyl ether, >500, ethyl acetate, 500; hexane, >500; methanol, >500; toluene, >500 (all g/L)

Partition coefficient: *n*-octanol/water ($logP_{ow}$) at 20 °C = 6.9

Relative density: 1.25 (25 °C)

Refractive Index: $n_d^{24} = 1.534$

Ultraviolet maxima: not reported

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Cyhalothrin is a synthetic pyrethroid insecticide used in many countries on various food-producing animals for the control of a broad range of ectoparasites, including flies, lice and ticks. Major use is on cattle and sheep, with lesser use on pigs and goats.

Typical product formulations include a 20 g/L liquid intended for direct dermal application as a "pour-on" or "spot-on", and emulsifiable concentrate formulations containing from 16 g/L to 200 g/L cyhalothrin, intended for use as a spray or dip after dilution with water. Cyhalothrin is applied topically as a pour-on at up to 60 ml (1.2 g) for ticks and at 10 ml (0.2 g) for lice or flies on cattle and at 5 ml (0.1g) on sheep and pigs for all applications. Cyhalothrin is also available as a 20% (w/v) emulsifiable concentrate for use as a spray or a dip prepared by dilution to 0.002-0.2% and applied at a dose of 0.1-4 L per animal, depending on the size of animal and the pest for which it is applied. The recommended repeat treatment intervals are 2-4 weeks for the spray or dip, and 4-8 weeks for the pour-on formulations. More frequent applications may be required for the control of ticks.

PHARMACOKINETICS AND METABOLISM

Laboratory Animals

Rat

Excretion

Groups each consisting of 6 male and 6 female rats (200-250 g BW) received single oral doses of ^{14}C-labelled cyhalothrin at 1 mg/kg and 25 mg/kg, labelled at two different positions, the 1-position of the cyclopropane ring (cyclopropyl-label) or the α-carbon of the benzyl group, or benzyl-label (Harrison, 1981a,b The radiolabelled cyhalothrin was dissolved in corn oil for administration to animals that had fasted overnight. Excreta were collected from each rat at 24-hour intervals for 6 to 7 days after exposure, following which the animals were euthanized and selected tissues were collected for measurement of residual radioactivity.

Radiolabelled residues were rapidly excreted in urine and faeces following all treatments, with most of the benzyl-labelled cyhalothrin dose was excreted within 24 hrs post treatment (Harrison, 1981a). Monitoring of expired air from rats (2 males, 2 females) dosed orally at 1 mg/kg did not reveal any elimination via this route. Different patterns of urinary metabolites were observed following treatment with cyhalothrin labelled at each of the two positions, with extensive metabolism and excretion of polar metabolites in the urine was determined by thin-layer chromatographic analysis. No significant difference in elimination rate or route was observed between males and females at either dose, or with either label. Urine contained 30.0-41.5% of the excreted radiolabel in all groups treated with the benzyl-label, while the balance of each dose was in the faeces. Following treatment with the cyclopropyl-label cyhalothrin, excretion was slower at the high dose than with the benzyl-label, with only 20-30% of the total radioactivity eliminated in the first 24 hrs. Overall, less radiolabel product was excreted in the urine from the rats treated with the cyclopropyl-label material (18.6–39.3%). Much of the radiolabel in the faeces was unchanged parent compound, indicating incomplete absorption. Only 1-3% of the dose remained in animal tissues at 7 days after exposure, predominantly in the fat.

In the same study, other rats received a single subcutaneous injection of 1 mg/kg benzyl-labelled cyhaothrin. Excretion was slower than from oral dosing, with 58% of the dose remaining in the tissues after 7 days. This was attributed to retention of the oil formulation in the subcutaneous fat. This study also included a treatment of bile duct canulated rats in which the benzyl-labelled cyhalothrin was administered orally at 1 mg/kg. Biliary excretion accounted for only 4.8–8.9% of the radiolabel residues, while urinary excretion was 7.2–8.3%, much less than observed in intact animals, but this increased to 16.8% in urine and 11.2% in bile when bile duct canulated rats were co-administered bile with the oral cyhalothrin dosing. The results suggested that bile strongly influenced the digestion process. Peak concentrations of cyhalothrin were observed in blood at 4-7 h after oral dosing for the various treatments and it depleted exponentially, with a half-life of approximately 11 h. This study report did not include a statement of GLP compliance.

Three studies contained GLP compliance statements. In one report on residues in tissues from rats included in the oral dosing study described above, residues were over 10-fold higher, as expected, in tissues from animals that received the 25 mg/kg dose and residues were detectable in all tissues, including brain, bone, fat, heart, liver, lungs, kidney, muscle and spleen from all rats at both doses (Harrison, 1981b). Residues were also detected in blood and bone. Residues in white fat varied from 0.17 to 0.34 mg/kg for the four treatment groups (males and females, 2 label positions) that received the 1 mg/kg dose and from 6.41-11.84 mg/kg for those which received the 25 mg/kg dosing. There was no significant difference in residue distributions between males and females, or between the residues in fat resulting from treatment with cyhalothrin labelled at the different positions. Residues in other tissues were much lower (>100-fold difference at 25 mg/kg treatment).

Rats (divided into two groups, each consisting of 6 males and 6 females) received 0.5 mL of a corn oil solution containing 0.5 mg/mL of either benzyl-labelled or cyclopropyl-labelled ^{14}C-cyhalothrin, administered orally by gavage tube, once daily for 14 days (Harrison, 1984a). The rats were kept in metabolism cages and urine and faeces were collected for each 24-hour period until the animals were killed at intervals up to 7 days after the last dose. Two males and two females from each group were killed at each of 48 hrs, 120 hrs and 7 days after the last dose and tissue samples were collected for analysis. Over 90% of the dose was eliminated in urine and faeces within 7 days after the final administration, with <5% remaining in the carcasses. Overall recovery of the total radioactive dose was 96% for both males and females and for both labelled forms of cyhalothrin. While elimination was rapid for both labelled forms, there were significant differences in the distribution of radioactive residues in urine and faeces, depending on the site of labelling. Residues in the tissues tested were primarily in fat, as parent compound. The estimated half-life for the residues in fat was 23 days. The distribution and elimination observed for cyhalothrin in this multiple dosing study was similar to that observed in the previous single dose study.

Metabolism

In one study, rats were administered ^{14}C-cyhalothrin (labelled at the benzyl-position) orally for 8 days, so that each animal received a total dose of 25 mg. Approximately 64% was recovered in the urine (Harrison, 1983). Urine was also collected and pooled from 14 rats that received 14 consecutive daily doses (1 mg/kg/day) of cyclopropyl-labelled ^{14}C-cyhalothrin. ^{14}C-components in urine from the two dosing experiments were separated by thin-layer chromatography (TLC) and analysed by high performance liquid chromatography (HPLC) using a radiochemical detector in combination with a uv-detector. Spectral identification techniques used included electron impact mass spectrometry, fast atom bombardment mass spectrometry (FAB-MS) in both positive and negative ion modes and ^{13}C-nuclear magnetic resonance (NMR). TLC analysis of urine from animals dosed with the benzyl-labelled ^{14}C-cyhalothrin indicated the presence of one major metabolite, designated M1, that accounted for about 75% of the radioactivity in urine. Three minor metabolites, designated M2, M3 and M4 were also observed, of which the least polar, M2, co-chromatographed with a standard of 3-phenoxybenzoic acid in the two solvent systems used for separation. No parent compound were observed. Further analysis by TLC again indicated that the least polar component from each urine sample co-chromatographed with M2. The fractions containing M2 were pooled and concentrated for mass spectral analysis using both direct inlet mass spectrometry and GC/MS after derivatization, with both techniques confirming the identification of M2 as 3-phenoxybenzoic acid. Following further separation of the remaining fractions by HPLC, one major peak, identified as M1 by TLC, and two minor peaks (<5%) were separated. The M1 was hyrolyzed by incubation with aryl sulphatase enzyme to a single component that co-chromatographed with M3. Treatment of M1 with β-glucuronidase resulted in only about 10% hydrolysis. Structural examination by NMR of purified M1 and its hydrolysis product indicated that the latter is phenol 3-(4'-hydroxyphenoxy) benzoic acid, while M1 is the sulphate conjugate. Urine from rats treated with the cyclopropyl-labelled ^{14}C-cyhalothrin was shown by TLC to contain one major ^{14}C-component (about 50%) and four minor components. The major component was completely hydrolyzed using β-glucuronidase, resulting in a corresponding increase in MII, one of the minor metabolites. This material co-chromatographed with the acid moiety of cyhalothrin, (1*RS*)cis-3-(*Z*-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid (CPA). Using HPLC and TLC, a main fraction was separated that contained the major ^{14}C-cyclopropyl metabolites. The main metabolite, MIII, accounting for 55% of the residual radioactivity, was purified by additional HPLC and examined by electron impact mass spectrometry and by FAB-MS, confirming that it was a glucuronide conjugate of MII.

Dog

Excretion

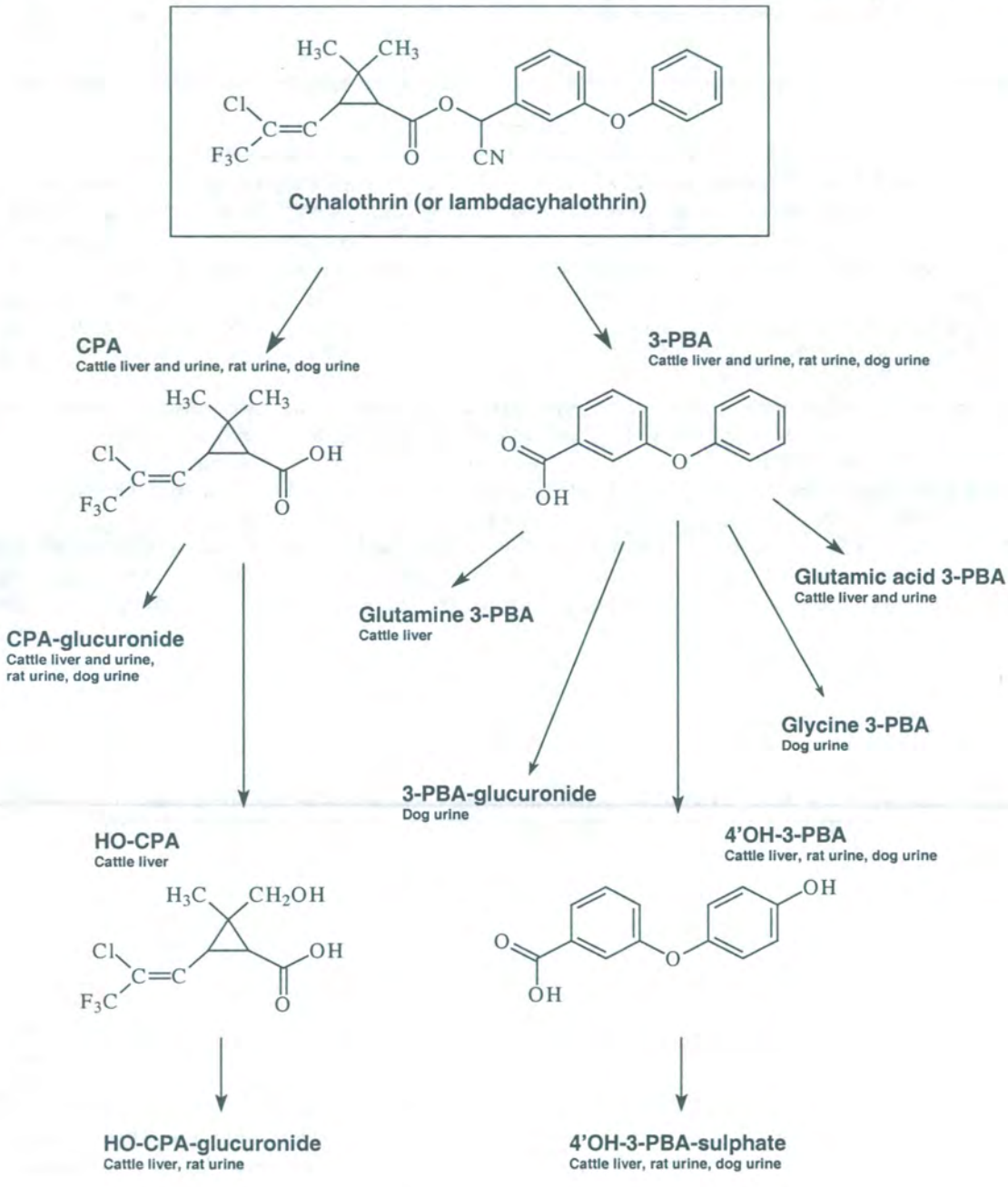
A series of studies were conducted in beagle dogs (3 males, 3 females, each approx. 15 kg BW), dosed orally (1 and 10 mg/kg BW) and intravenously (0.1 mg/kg) with benzyl-labelled and cyclopropyl-labelled ^{14}C-cyhalothrin (Harrison, 1984b). In each dosing regimen, each animal was dosed twice, once with each labelled form of cyhalothrin, at the chosen dose, in the following order: 1 mg/kg oral, 10 mg/kg oral, 0.1 mg/kg intravenous; benzyl-label first, cyclopropyl-label second in the oral studies; the reverse in the intravenous study. At least three weeks elapsed between dose treatments. The dogs were individually housed in metabolism cages for sample collection. Urinary metabolites were separated by TLC and detected by autoradiography. Spectral identification was by electron impact mass spectrometry or FAB-MS. After oral administration of the benzyl-label drug, most of the radiolabel was excreted within 48 hrs, with 65-70% in the faeces and 16-20% in the urine. Oral administration of the cyclopropyl-label drug gave a similar overall result, but with 46-55% of the radioactive residue in the faeces and 25-40% in the urine. After administration of the cyclopropyl label drug, peak concentrations occurred in the blood at 4 and 12 hrs, respectively, for the 1 and 10 mg/kg doses. With the benzyl-label drug, peak concentrations in blood were at 2-12 hrs for 1 mg/kg and at 12 hrs for 10 mg/kg doses, with half-lives of 28 and 32 h, respectively. No significant differences in absorption or elimination were observed between the sexes. Overall, from 79-96% of the dose was accounted for in the urine, faeces and cage washings. Excretion was slower after intravenous dosing, but was largely complete within three days, with similar distribution of residues in urine (36-44%) and faeces (34-43%), irrespective of label position.

Metabolism

Twelve urinary metabolites were detected following oral or intravenous administration, with the major components being CPA and its glucuronide. Other urinary metabolites identified are shown in Figure 1. Parent compound was the main residue in the faeces following oral administration, but little parent compound was found in faeces after intravenous administration. This study included a statement of GLP compliance.

Results of these metabolism studies are shown in Figure 1.

Figure 1 **Metabolic pathway for cyhalothrin (or lambda-cyhalothrin) in rats, dogs and cattle. (Some identifications are tentative. Metabolism by goats is similar)[a]**



[a] Figure provided courtesy of Sponsor.

Food Producing Animals

Cattle

Metabolism

Two lactating cows were given ^{14}C-labelled cyhalothrin orally to determine the metabolism and distribution resulting from a treatment corresponding to the total dose received in a typical dermal application (Harrison, 1984c). Each cow was dosed orally twice daily at 1 mg/kg bw/day for 7 days, one with benzyl-labelled cyhalothrin, the second with cyclopropyl-labelled cyhalothrin. Concentrations of cyhalothrin in blood rose rapidly for the first 50 hrs of the study, then slowly for the remainder of the study. Maximum concentrations of 0.16 and 0.27 mg eq./L, respectively, for the benzyl-label and cyclopropyl-label drug. An equilibrium was observed between ingestion and excretion within 3 days of the start of dosing. Overall, 76-77% of both the benzyl-label and the cyclopropyl-label cyhalothrin residues were accounted for- 49% in faeces, 27% in urine; 0.6% in pen wash; and 0.8%in milk, when the animals were slaughtered 16 hours after the final dose. Parent compound was the major residue in faeces, while CPA glucuronide was the major urinary metabolite from the cyclopropyl-labelled cyhalothrin. The major urinary metabolite from the benzyl-label cyhalothrin was tentatively identified by TLC as the glutamic acid derivative of 3-phenoxybenzoic acid (3-PBA). Parent compound was 11-28% of the residue in kidney, but only 3% of the total residue in liver. CPA and its glucuronide were major metabolites identified from the cyclopropyl-label ^{14}C-cyhalothrin in both liver and kidney. These and other metabolites (some only tentatively) are shown in Figure 1. Residues in milk were determined daily during the study and residues in fat, liver, kidney and muscle were determined at slaughter. Highest total residues of cyhalothrin found in the tissues, resulting from the treatment with the cyclopropyl label, were: perirenal fat, 2.69 mg/kg; subcutaneous fat, 1.61 mg/kg; liver, 1.28 mg/kg; kidney, 0.60 mg/kg; muscle, 0.19 mg/kg. In milk, the highest residue observed was 0.59 mg/kg, from the afternoon milking on the final day of dosing with the cyclopropyl label. All the radiolabel in milk partitioned into the cream, with parent compound accounting for 96% and 88% of the total residues from treatment with the cyclopropyl- and benzyl-label ^{14}C-cyhalothrin, respectively. Parent compound accounted for 85% and 91% of the total residues in fat associated, respectively, with the cyclopropyl- and benzyl-label. drug. The usual 60:40 ratio of A:B isomers was essentially reversed in the perirenal fat and in milk, suggesting that the B isomers are more persistent than the A isomers. The study was GLP compliant.

In another study, two lactating cows were given 15 mL of a pour-on formulation of ^{14}C- lambda-cyhalothrin {*RS*-α-cyano-3-phenoxybenzyl (1*RS*) –*cis*-3-(*Z*-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate} (containing a 1% benzyl- label) in maize oil and volatile silicone, once daily for 3 days (Dow, et al, 1989). Application was as a streak down the midline of the back and followed the morning milking. The animals were killed after the morning milking, 24 hours after the third application of the formulation. As in the oral dosing study with ^{14}C-cyhalothrin discussed above, the residues in milk were predominantly parent drug (87-113%). The maximum residue found in milk was 0.16 mg/kg, in the final milking. In fat, 80-113% of the total residue was parent compound, while residues in muscle were 80-88% parent compound. Seven components were separated from liver, with parent compound comprising less than 5% of the total residue and the remainder attributed to metabolites or unassigned. At least 6 components were separated in kidney, with parent compound accounting for up to 20% of the total radioactive residue. The main metabolite identified was 3-PBA, accounting for 7% of the total residue in liver and 8% in kidney. Maximum total residues found in tissues were: perirenal fat, 0.15 mg/kg; subcutaneous fat, 0.21 mg/kg; omental fat, 0.12 mg/kg; liver, 0.31 mg/kg; kidney, 0.13 mg/kg; and in muscle, 0.03 mg/kg. This study was also GLP compliant.

Further work was done to identify three unidentified metabolites, designated L1, L2 and L3, separated in the previous study (Dow & Parker, 1989). Following fractionation by HPLC, the metabolites were methylated and studied using mass spectrometry (electron impact direct probe, FAB-MS and GC/MS), in comparison with reference compounds. L3, which accounted for 25% of the total residue in liver, was identified as a glutamic acid conjugate of 3-PBA. Mass spectral data also suggested that L1 (2% of the total residue) was a 3-PBA conjugate. No identification of L2 (11% of the total residue) could be made from the mass spectral data, but the chromatographic behavior suggested a similar structure to L1 and L2. Metabolites L1 and L3 could be analyzed as 3-PBA following acid hydrolysis of samples.

In a related study, two lactating cattle were treated according to the same protocol as described above with 1% cyclopropyl-labelled ^{14}C-lambda-cyhalothrin in maize oil and volatile silicone (Knight et al, 1989). Excreta were not collected in this study. The maximum residue found in milk was 0.15 mg/kg, in the final milking. Maximum total residues found in tissues were: perirenal fat, 0.13 mg/kg; subcutaneous fat, 0.07 mg/kg; omental fat, 0.08 mg/kg; liver, 0.23 mg/kg; kidney, 0.14 mg/kg; muscle, 0.03 mg/kg. Parent compound was the major residue in milk, accounting for 92-100% of the total residue. Parent compound was 85-92% of the total residue in muscle, 10% in liver, 37% in kidney and 54-77% in muscle. The major metabolites in liver and kidney were CPA, hydroxy-CPA and their conjugates.

Goats

A single lactating goat (50 kg bw) was dosed orally at a concentration equivalent to 10.8 mg/kg in the total diet, twice daily at milking for 7 days, with cyclopropyl-labelled ^{14}C-lambda-cyhalothrin formulated in gelatin capsules (Leahey et al, 1985). Milk, urine and faeces were collected throughout the dosing period and until the goat was killed at 16 hrs

after the final dose was administered. No blood was tested in this study. Distribution of total residues was similar to that observed in cattle, with highest residues in perirenal fat (0.44 mg/kg), followed by liver (0.34 mg/kg), omental fat (0.33 mg/kg), kidney (0.20 mg/kg), subcutaneous fat (0.13 mg/kg) and muscle (0.024–0.028 mg/kg). Parent compound accounted for 89% of the total residue in fat, 94% of the total residue in muscle and 6% of the total residue in liver. CPA was the major metabolite in liver (21%, mainly unconjugated) and in kidney (58%, mainly conjugated). Total residues in milk, mainly as parent drug, were from 0.005 mg/kg after the first dose to a maximum of 0.27 mg/kg on day 5, then declining to 0.17 mg/kg in the final sample collected. This report included a statement of GLP compliance.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

No depletion studies using radiolabeled cyhalothrin were reported.

Residue Depletion Studies with Unlabelled Drug

The studies reported in this section were all conducted according to GLP requirements.

Cattle

Twenty-two calves (105-165 kg) were randomly divided into 4 treatment groups (5 animals per group), two control animals (Bicknell et al, 1986) . Each of the animals in the treatment groups received a cyhalothrin ear tag-tape on day 0. In addition, the calves in the treatment groups were sprayed on day 0 with a solution containing 500 mg/L cyhalothrin, prepared from a 20% emulsifiable concentrate with water, corresponding to a dose of about 2 mg cyhalothrin/kg body weight per treatment. Spray treatments were repeated at 14 day intervals until each animal had received a total of 7 treatments (day 84). Treatment groups were then slaughtered at 0, 3, 7 and 14 days following final treatment. The two control animals were maintained and slaughtered separately from the treated animals. Samples of perirenal and subcutaneous fat, liver, kidney and muscle were collected from all carcasses, but only analyses of fat were reported in this study. The analytical method used gas chromatography with electron capture detection, with recoveries of 83% and a limit of detection (LOD) of 0.003 mg/kg. Residues, corrected for recovery, are given in Table 1. Residues of cyhalothrin from the animals in the day 0 withdrawal group, not corrected for recovery, were: 0.005 ± 0.003 mg/kg in liver; 0.010 ± 0.003 mg/kg in kidney; and 0.005± 0.001 mg/kg in muscle(Bicknell et al, 1989a).

Table 1. Residues in cattle fat (mg/kg) using eartags containing cyhalothrin and 7 weekly spray applications (approximate dose of 2 mg/kg body weight per treatment).

	Cyhalothrin residues (mg/kg), n=4	
Withdrawal time (days)	Subcutaneous fat	Perirenal fat
Control	<0.003	<0.003
0	0.26 ± 0.12	0.32 ± 0.13
3	0.15 ± 0.04	0.17 ± 0.07
7	0.19 ± 0.05	0.21 ± 0.05
14	0.12 ± 0.02	0.16 ± 0.03

Twenty-four heifers (250-350 kg BW) were randomly divided into four groups of five animals and two groups of two animals (Bicknell et al, 1987). The four 5-animal groups were each treated with 30 mL of a formulation containing 2% cyhalthrin in maize oil and volatile silicone, applied by syringe along the back, on days 1, 7, 14 and 21. This represents a dose of about 2 mg cyhalothrin/kg BW. In the remaining groups, two animals were treated with 60 mL of the cyhalothrin formulation (about 4 mg cyhalothrin/kg BW) as described, while the second group of two animals was segregated on a separate farm as controls. The control animals were slaughtered separately on day 21. One group of 5 heifers, plus the 2 animals that received the doubled dose of cyhalothrin, were slaughtered at 5-7 h. after the final treatment. The remaining groups of 5 heifers were slaughtered at 3, 7 and 14 days after the final treatment. Samples of subcutaneous and perirenal fat, kidney, liver and quadriceps muscle were collected at slaughter, but only fat and liver were analyzed by gas chromatography with electron capture detection. Mean recoveries on fortified fat samples were 78% at 0.1 and 0.6 mg/kg using the analytical procedure described for fat. The assay for liver gave a mean recovery of 82% at 10-95 mg/kg. The limit of quantitation (LOQ) was 0.01 mg/kg for both fat and liver. The results reported in Table 2 were corrected for recovery.

Table 2. Residues in fat and liver of heifers treated with 4 weekly applications of a 2% cyhalothrin formulation.

Withdrawal period	Treatment	Cyhalothrin Residue Concentration (mg/kg), n=4		
		Subcutaneous fat	Perirenal fat	Liver
Control	None	<0.01	<0.01	<0.01
7 hrs.	30 mL	0.67 ± 0.30	0.91 ± 0.37	<0.01
7 hrs.[1]	60 mL	0.57, 0.68	0.94, 1.14	<0.01, 0.01
3 days	30 mL	0.61 ± 0.14	0.87 ± 0.24	<0.01
7 days	30 mL	0.48 ± 0.09	0.64 ± 0.14	*
14 days	30 mL	0.35 ± 0.17	0.91 ± 0.55	*

Note. [1] only 2 animals in this treatment group;* = not analyzed.

Five dairy cattle (320-392 kg BW) each received two tag-tapes containing cyhalothrin, attached to an ear-tag, while an additional five cattle (260-422 kg BW) were each sprayed 7 times, at 14-day intervals, with a spray solution at about 2 mg cyhalothrin/kg BW (Knight et al, 1985). Milk samples were collected from both groups of animals throughout the study and analyzed by a gas chromatographic method using electron capture detection (LOQ of 0.005 mg/kg, LOD of 0.001 mg/kg and recoveries of 89%). Control milk samples were collected from the animals prior to treatment and from untreated animals. Control samples from the animals selected for treatment with the tag-tapes were estimated to contain approximately 0.002 mg/kg cyhalothrin prior to treatment. 105 milk samples were collected and analyzed following application of the tag-tapes with only 9 of these samples having residues in excess of the LOQ (0.005 mg/kg). The highest residue observed in a milk sample from this treatment group was 0.012 mg/kg. The majority of the 150 milk samples collected and analyzed from the group treated by spray application contained cyhalothrin residues in excess of 0.005 mg/kg, but only 15 samples exceeded 0.01 mg/kg, with the highest concentration found being 0.018 mg/kg. Results were not corrected for recovery in this study.

Five dairy cattle (260-435 kg BW) were each sprayed with 4 liters of an aqueous solution corresponding to a dose of about 0.4 mg cyhalothrin/kg body weight (Bicknell et al, 1989a). These animals were those used in the previous study that received 7 spray treatments, (Knight et al, 1985). There was a 7-day interval between the two studies. The 0.4 mg/kg BW treatment was repeated at 7-day intervals for four spray applications. The animals were slaughtered at zero withdrawal. Milk was collected twice daily during this study and stored in a freezer (-15 to -20 °C) until analyzed. Milk and tissue samples were analyzed by gas chromatography with electron capture detection. (recoveries were 89% for residues in milk, with an LOD of 0.001 mg/kg; in tissues, recoveries ranged from 82% in fat and kidney to 84% in liver and 100% in muscle, with corresponding LODs of 0.005-0.01 mg/kg for the various tissues). Residues in tissues were: 0.10 ± 0.03 mg/kg in perirenal fat; 0.16 ± 0.22 mg/kg in subcutaneous fat; <0.01 mg/kg liver, kidney and muscle: Overall, mean concentrations of cyhalothrin in milk ranged from 0.005 ± 0.001 to 0.008 ± 0.003 mg/kg at the first milking following a spray application and from 0.004 ± 0.001 to 0.008 ± 0.002 mg/kg at the 14th milking . Residues in milk typically peaked at 3-4 days following a spray application, with a maximum mean value of 0.013 ± 0.004 mg/kg observed at the 6th milking, 3 days after the third spray application.

In another study, four dairy cattle (480-535 kg BW) received 4 treatments, at 7-day intervals, of a pour-on formulation containing 2% cyhalothrin in maize oil and volatile silicone, or a dose of about 1.2 mg cyhalothrin/kg BW (Knight et al, 1987a). Two other dairy cattle (415 and 495 kg BW) received the same series of treatments, but with 3.6 mg cyhalothrin/kg BW (treatment 1) followed by three weekly treatments of 2.4 mg cyhalothrin/kg BW (treatments 2-4), of the pour-on product. Control milk was collected from all the animals in the two treatment groups for four days prior to the first administration of pour-on, with additional control milk obtained from untreated animals. Samples were collected at each milking for 35 days following the initial application of cyhalothrin and stored frozen until analyzed. Analysis was by gas chromatography with electron capture detection, similar to that reported in the previous study (Knight et al, 1985). Validation work for this study indicated recoveries of 97 ± 21%, with an LOD of 0.001 mg/kg and an LOQ of 0.009 mg/kg. The Highest residue concentrations were observed at the 3-5 milkings following application of the 1.2 mg/kg BW treatment, with a maximum observed value of 0.31 mg/kg. At this treatment rate, residues were consistently <0.005 mg/kg by the 7th milking following final application. In milk from the cows that received the over-dose treatments, the highest residue observed in milk was 0.47 mg/kg on day 3 following the application of 3.6 mg/kg BW of the product, while the highest residue observed with the 2.4 mg/kg BW formulation was 0.30 mg/kg at the third milking following the first application. Highest residues were observed in each case from 3-7 milkings post-treatment and did not remain below 0.005 mg/kg until 16 milkings after final treatment.

In a subsequent study, five dairy cattle (470-590 kg BW) were treated with a single application of a 2% pour-on solution of cyhalothrin in maize oil and volatile silicone (1.2 mg cyhalothrin/kg BW), applied directly along the mid-line of the back (Bicknell et al, 1989b). Two other cattle served as controls. Samples were collected for analysis at each of the twice-daily milkings for 7 days following treatment and analyzed as in previous studies. Highest residue concentrations of cyhalothrin were found in milkings 3-5 post-treatment, at concentrations ranging from 0.02 – 0.07 mg/kg, falling to <0.01 mg/kg by day 7.

Pig

Twelve healthy pigs (39-50 kg BW) each received a single application of a formulation containing 2% cyhalothrin in maize oil and volatile silicone, administered by syringe as a single streak about 5 cm long on the back of the neck (Bicknell et al, 1988). This corresponds to a dose of about 3 mg cyhalothrin/kg BW. The pigs were randomly divided into 3 groups of 4, then slaughtered at 3, 7 and 14 days after treatment, respectively. Four other pigs served as controls and were slaughtered prior to application of the cyhalothrin. Tissue samples were collected at slaughter and analyzed by gas chromatography with electron capture detection (LOD, 0.001 mg/kg; LOQ, 0.010 mg/kg; recovery 75-97%, depending on tissue). Analytical results were corrected for recovery and are summarized in Table 3.

Table 3. Residues of cyhalothrin in pigs treated with a single dermal application of 2% cyhalothrin in maize oil and volatile silicone to the neck.

Withdrawal Time (days)	Mean concentration of cyhalothrin (mg/kg), n=4						
	Liver	Kidney	Muscle	Subcutaneous fat	Abdominal fat	Skin (flank)	Skin (Application site)
Controls	<0.01	<0.01	<0.01	<0.01	na	<0.01	na
3	<0.01	<0.01	<0.01	0.08 ±0.05	0.08 ± 0.10	0.23 ± 0.04	0.82 ± 0.44
7	-na	na	na	0.04 ±0.02	0.05 ± 0.04	0.15 ± 0.03	0.35 ± 0.20
14	na	na	na	<0.01	0.04 ± 0.01	0.09 ± 0.08	0.33 ± 0.13

Note. na, means not analyzed.

Sheep

Fifteen sheep (28-36 kg BW) were randomly divided into 5 groups of 3 each. One group served as controls and sheep in the remaining 4 groups each received 3 applications of cyhalothrin in maize oil and volatile silicone, at 14 day intervals, administered by syringe directly onto the skin down the midline of the back (Knight et al, 1987b). This corresponds to a dose of about 2.25 mg cyhalothrin/kg BW. The treated animals were killed in groups at 16 h, 3, 7 and 14 days after the third application and tissue samples were collected for analysis. The animals in the control group were killed prior to the commencement of the treatments. Analysis was by gas chromatography with electron capture detection ((LOD, 0.001 mg/kg; LOQ, 0.01 mg/kg for fat, kidney, muscle, and 0.05 mg/kg for liver; recovery 78-84%, depending on tissue). Residues were measured in tissues and are reported in Table 4.

Table 4. Residues in sheep administered 3 treatments of a pour-on formulation of 2% cyhalothrin in maize oil and volatile silicone.

Withdrawal time	Cyhalothrin residues found in tissues (mg/kg), n = 3				
	Liver	Kidney	Muscle	Subcutaneous fat	Perirenal fat
Controls	<0.05	<0.01	<0.01	<0.01	<0.01
16 hrs	<0.05	<0.01	<0.01	0.04 – 0.13	0.03 – 0.08
3 days	<0.05	<0.01	<0.01	0.03 – 0.05	0.03 – 0.05
7 days	<0.05	<0.01	<0.01	0.03 – 0.08	0.06 – 0.08
14 days	<0.05	<0.01	<0.01	<0.01	0.04 – 0.10

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

The sponsor has provided an analytical method originally developed for the analysis of fat, with extensions to other tissues, based on gas chromatography with electron capture detection (Knight and Parker, 1988). A 20 g fat sample is mixed with 5 g anhydrous sodium sulphate and acetone-hexane (1:1) and homogenized, warmed in a water bath at 60°C to dissolve the fat, then partitioned with water. Following solvent partition the hexane extract is transferred to a cyano-bonded SPE cartridge, and cyhalothrin is eluted with 10% THF in hexane. Analyis is by gas chromatography using a 1.5 m x 4 mm id column packed with 5% OV-101 on 60-80 mesh Gas Chrom Q, with nitrogen carrier gas at 20 mL/min flow. The recommended injection volume is 5 µL. A typical retention time for cyhalothrin under these conditions is 2.8 min. Muscle samples are also analyzed using this procedure.

For liver, the method is modified by adding 5 g celite and 20 g anhydrous sodium sulphate to the initial 20 g sample of liver. Following homogenization with acetonitrile, the sample is further purified on a florisil column and analyzed by

gas chromatography, as above. For kidney samples, the procedure for fat is followed to the column clean-up step, then the clean-up using the florisil column for liver is used.

A confirmatory procedure has also been described for residues of cyhalothrin in fat. Following the purification for gas chromatographic analysis, an additional clean-up step is added using a florisil column, prepared as described in the procedure for liver analysis. The final extract is added to the florisil column and eluted. The GC/MS analysis is conducted using a 25 m x 0.32 mm id fused silica column coated with SE54, or equivalent, with a helium flow rate of 1 mL/min. Sample injection is at near-ambient temperature, using an oven temperature . The ion fragments detected are m/z 197, 199 and 208.

Similar methodology has been reported for the analysis of milk samples (Bicknell et al, 1989a). Whole milk (100 mL) is freeze-dried and extracted with hexane. Cyhalothrin is then partitioned into DMF and 1% aqueous sodium sulphate is added to the DMF, after which the mixture is again extracted with hexane. Following solvent partition, this residue eluate is transferred to a cyano-bonded SPE cartridge as described above in the procedure for fat. Conditions for gas chromatographic analysis are as described for fat samples.

Variations on the methodology have been used in the various depletion studies reported by the sponsor, with method performance tests included in each report. The performance of the GC methodology used for various matrices in these studies has been summarized in Table 5.

Table 5. Method performance characteristics of the gas chromatographic methods for various sample matrices as reported in depletion trials.

Species	Matrix (Tissue or Milk)	Analytical Range (mg/kg)	Recovery (%)	LOD* (mg/kg)	LOQ** (mg/kg)	Precision over Analytical Range (%)
Bovine	Milk	0.01–0.05 to 0.01–0.47	74–97	0.001	0.01	8–25
	Liver	0.01–0.11	82–84	0.003–0.005	0.01	7–21
	Kidney	0.01–0.05	82	0.005	0.01	12
	Muscle	0.01–0.05	100	0.005	0.01	9
	Fat	0.01–0.62	78–83	0.001–0.003	0.01	11–13
Porcine	Liver	0.01–0.60	97	0.001	0.01	6
	Kidney	0.01–0.60	86	0.001	0.01	10
	Muscle	0.01–0.60	80	0.001	0.01	10
	Fat	0.01–0.60	89	0.001	0.01	11
	Skin	0.01–0.60	75	0.001	0.01	10
Ovine	Liver	0.05–0.09	84	0.001	0.05	5
	Kidney	0.01–0.09	79	0.001	0.01	9
	Muscle	0.01–0.09	84	0.001	0.01	12
	Fat	0.01–0.40	78	0.001	0.01	32

*Determined statistically from linear regression approach using control and fortified samples.
** The lowest concentration at which recovery and precision were investigated.

More recently, a method for the analysis of cyhalothrin residues in milk using negative ion chemical ionization gas chromatography-mass spectrometry (GC/MS-NICI) has been proposed (McCormack, 1999a). A 1 g sub-sample of milk is mixed with 20 mL acetonitrile using ultrasonication for 10 min, then left to stand in a water bath at 50°C for 15 min. The homogenate is centrifuged and the supernatant liquid is decanted. The extract is washed with 20 mL hexane. The acetonitrile solution is reduced to <10 mL at 40 °C and cleaned up on a C-18 solid phase extraction cartridge. The eluate from the cartridge containing cyhalothrin is further cleaned up using a florisil cartridge. GC/MS analysis is conducted using a 30m x 0.32 mm id CP-SIL 8 column (0.25 µm film thickness. A flow rate of 2 mL/min helium (20:1 v/v split) is used, with methane as reagent gas. The sample injection volume is 3 µL. Cyhalothrin is detected as two pairs of enantiomers, at 11.6 and 11.8 min, monitored as the fragment ion m/z 205. The method also has been applied to bovine muscle, liver, kidney and fat, with variations in the clean-up procedure according to the tissue analyzed. In a second report, the method was applied to ovine muscle, liver, kidney and fat (McCormack, 1999b). Performance characteristics of the GC/MS-NICI method for various matrices are given in Table 6.

Table 6. Performance characteristics reported for GC/MS-NICI determination of cyhalothrin residues in bovine milk and tissues and ovine tissues.

Species	Matrix (Tissue or Milk)	Analytical Range (mg/kg)	Recovery (%)	LOD* (mg/kg)	LOQ** (mg/kg)	Precision at LOQ (%)
Bovine	Milk	0.01–0.04	96	0.004	0.01	18
	Liver	0.02–0.10	95	0.02	0.02	24
	Kidney	0.02–0.10	88	0.02	0.02	20
	Muscle	0.02–0.10	80	0.02	0.02	24
	Fat	0.25–1.0	75	0.05	0.25	8
Ovine	Liver	0.02–0.10	85	0.02	0.02	28
	Kidney	0.02–0.10	97	0.01	0.02	20
	Muscle	0.02–0.10	97	0.02	0.02	22
	Fat	0.25–1.0	100	0.17	0.25	32

*Determined statistically from linear regression approach using control and fortified samples.
** The lowest concentration at which recovery and precision were investigated.

APPRAISAL

Cyhalothrin is a synthetic pyrethroid insecticide comprised of of two pairs of enantiomers, designated **A** and **B**, in an approximately 60:40 ratio (**A**:**B**). This ratio is usually reversed in residues found in fatty tissues. The product is intended for dermal application as a pour-on, dip or spray, with some potential oral intake from treated animals through grooming. Cyhalothrin may also be registered for horticultural uses and some oral ingestion may occur through feeds, but no specific information was provided on such exposure. Any MRLs assigned by JMPR, and their contribution to the TMDI, need to be considered in assigning MRLs for veterinary use. Cyhalothrin is also a compound with a long history of use that could be considered under JECFA provisions for such compounds.

Information was provided on metabolism and distribution in both laboratory animals (rats, dogs) and food animals (cattle, sheep, goat), with generally consistent results across species . Cyhalothrin is excreted via urine and faeces, with <5% of the total dose remaining as a residue in treated animals. The highest residues of parent compound are found in fat, with concentrations generally being higher in renal fat than in other fatty deposits. Residues are much lower in kidney and muscle and are also primarily parent compound. Cyhalothrin is excreted in milk as parent compound and is associated with the fatty constituents of the milk. Metabolism occurs primarily in the liver, where radiolabel studies indicate the highest concentrations of total residue, but very little parent compound. Metabolism and distrtibution are similar to that observed for other synthetic pyrethroids Some of the studies on metabolism and distribution were conducted with lambda-cyhalothrin, a product with different toxicity but similar metabolism to cyhalothrin. It appears appropriate to use these studies to support the information gained in studies with cyhalothrin.

Adequate data were provided to recommend maximum residue limits (MRLs) for cattle, swine and sheep, where GLP studies with unlabelled compound were provided. The marker residue in all species is parent compound and fat (preferably renal) is recommended as the target tissue for testing, both in national residue control programs and for tissues in trade.

Performance data were provided for both GC and GC/MS determinative methods and for GC/MS confirmation of residues. While the methods require a relatively involved clean-up, they are well within the capabilities of a normally well-equippped residue laboratory.

Based on the data provided, MRLs can be assigned for edible tissues of cattle, swine and sheep, and for milk from cattle. The single metabolic study with radiolabelled lambda-cyhalothrin is not sufficient information on which to extend MRLs to goats.

MAXIMUM RESIDUE LIMITS

In considering the recommendation of Maximum Residue Limits, the Committee took into account the following:

- A temporary ADI of 0-2 μg/kg of body weight was recommended by the Committee, based on a toxicological endpoint, that allows for a maximum daily intake of 120 μg for a 60 kg person.
- The appropriate marker residue of cyhalothrin is the sum of the isomers, as previously established by JMPR.
- Cyhalothrin isomers account for less than 5% of the total residue found in liver of cattle and about 10 % or more of the total residue found in kidney. The metabolites are considered two-fold less toxic than the parent compound and

were accounted for using a factor of 16 for liver and 5 for kidney to adjust the marker residue to total residues in calculating the theoretical daily intake.

- Residues found in muscle, fat and milk are parent compound.
- The weekly application of the pour-on formulation to cattle and the volumes of this product used in excess of 10 mL leads to much higher residue concentrations in fat and milk than those found in the other residue depletion studies. While these uses may fall within the range of recommended applications for cyhalothrin, they were not considered as a suitable basis for the establishment of MRLs as they represent extreme uses. Use of the pour-on product can result in residue concentrations in excess of the MRL in milk.
- The maximum intakes assigned for horticultural use by JMPR account for 10% or less of the temporary ADI established by the Committee.
- A suitable analytical method is available for analysis of cyhalothrin residues in edible tissues and milk.
- MRLs for liver, kidney and muscle can be harmonized at twice the LOQ of the analytical method as validated for tissues from cattle and pigs.
- MRL recommended for fat are based on the highest mean residues, plus 3 standard deviations, as determined in depletion studies using treatments consistent with good practice in the use of veterinary drugs.
- The MRL recommended for milk is based on the highest mean residues, plus 3 standard deviations, as determined in depletion studies which used treatments with the spray formulation consistent with good practice in the use of veterinary drugs.

On the basis of the above considerations, the Committee recommended the following temporary MRLs for edible tissues of cattle, pigs and sheep, expressed as parent drug; muscle, 20 μg/kg; liver, 20 μg/kg; kidney 20 μg/kg; and fat, 400 μg/kg. The Committee also recommended a temporary MRL of 30 μg/kg for cyhalothrin in milk from cattle.

Based on consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat and 1.5 kg of milk and using the factors for liver and kidney as given above, the theoretical maximum daily intake of cyhalothrin residues from veterinary use is 108 μg. The remainder of the ADI has been allocated to other uses by the JMPR.

Table 7. Theoretical maximum daily intake (TMDI) of cyhalothrin residues

Food Item	MRL (μg/kg)	Food Basket (g)	μg	MR/TR[1]	TMDI (μg)
Muscle	20	300	6	1	6
Liver	20	100	2	1/16	32
Kidney	20	50	1	1/5	5
Fat	400	50	20	1	20
Milk	30	1500	45	1	45
Total:					108

Note: [1] MR = marker residue (parent drug); TR = total residues

REFERENCES

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1986). Residues of cyhalothrin in bodyfat of calves following multiple sprayings of 500 ppm cyhalothrin ex 20%E.C. and season long application of tap-tapes. Coopers Animal Health, Report CIBH 85-C4, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1987). Residues of cyhalothrin in bodyfat and liver of cattle following multiple treatment with a 2% cyhalothrin in maize oil and volatile silicone pour-on. Coopers Animal Health, Report CIBH 87-C4, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1988). Residues of cyhalothrin in the skin and tissues of pigs following treatment with a 2% cyhalothrin in maize oil and volatile silicone pour-on. Coopers Animal Health, Report CIBH 87-C5, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1989a). Residues of cyhalothrin in bovine milk and tissues following multiple sprayings of 250 ppm cyhalothrin ex 20%E.C. Coopers Animal Health, Report CIBH 85-C6, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1989b). Milk residues of cyhalothrin following a single application of a 2% pour-on. Coopers Animal Health, Report CIBH 89-1, Sponsor Submitted.

Dow, P.R., Knight, P.J., Parker, R.C., and Woollon, R.M. (1989). Metabolism of C-14 alcohol labelled lambda-cyhalothrin in lactating cows following dermal application. Coopers Animal Health, Report CIBH 88-4, Sponsor Submitted.

Dow, P.R., and Parker, R.C. (1989). Attempted identification of 3 major liver metabolites following dermal application of C-14 alcohol lambda-cyhalothrin to lactating cows. Coopers Animal Health, Report CIBH 89-2, Sponsor Submitted.

Harrison, M.P. (1981a). Cyhalothrin: the metabolism and disposition of carbon-14 labelled material in rats; Part I. Imperial Chemical Industries Report YIBH 85-C6, Sponsor Submitted.

Harrison, M.P. (1981b). Cyhalothrin (ICI 146,814): the metabolism and disposition of carbon-14 labelled material in rats; Part II. Tissue residues derived from carbon-14 benzyl or carbon-14 cyclopropyl labelled cyhalothrin after a single oral dose of 1 or 25 mg/kg. Imperial Chemical Industries Report YIBH 85-C7, Sponsor Submitted.

Harrison, M.P. (1983). Cyhalothrin:. the metabolism and disposition of carbon-14 labelled material in rats; Part IV. Isolation and identification of the major urinary metabolites derived from carbon-14 labelled benzyl or carbon-14 labelled cyclopropyl-cyhalothrin following oral administration. Imperial Chemical Industries Report YIBH 85-C9, Sponsor Submitted.

Harrison, M.P. (1984a). Cyhalothrin: the metabolism and disposition of carbon-14 labelled material in rats; Part III. Studies to determine radioactive residues in the rat following 14 days repeated oral administration. Imperial Chemical Industries Report YIBH 85-C8, Sponsor Submitted.

Harrison, M.P. (1984b). Cyhalothrin (ICI 146,814): the disposition and metabolism of carbon-14 labelled material in the dog. Imperial Chemical Industries Report YIBH 85-C10, Sponsor Submitted.

Harrison, M.P. (1984c). Cyhalothrin (ICI 146,814): the metabolism, excretion and residues in the cow after 7 days oral administration with two carbon-14 labelled forms of cyhalothrin at 1 mg/kg/day. Imperial Chemical Industries Report YIBH 85-C11, Sponsor Submitted.

Knight, P.J., Parker, R.C., Winter, R.L, and Woollon, R.M. (1985) Residues of cyhalothrin in bovine milk following multiple sprayings of 500 ppm cyhalothrin ex 20% E.C. or application of ear tag-tapes. Coopers Animal Health, Report CIBH 85-C3, Sponsor Submitted.

Knight, P.J., Mallard, K.L., Parker, R.C. and Woollon, R.M. (1987a) Residues of cyhalothrin in bovine milk following weekly application of a 2% cyhalothrin pour-on. Coopers Animal Health, Report CIBH 87-C3, Sponsor Submitted.

Knight, P.J., Parker, R.C., and Woollon, R.M. (1987b). Residues of cyhalothrin in sheep tissue following multiple treatment with a 2% cyhalothrin in maize oil and volatile silicone pour-on. Coopers Animal Health, Report CIBH 87-C2, Sponsor Submitted.

Knight, P.J, and Parker, R.C. (1988). A method for the analysis of cyhalothrin in animal tissues. Coopers Animal Health, Report CDBT 87-1, Sponsor Submitted.

Knight, P.J., Parker, R.C., and Woollon, R.M. (1989). Metabolism of C-14 acid labelled lambda-cyhalothrin in lactating cows following dermal application. Coopers Animal Health, Report CIBH 88-4, Sponsor Submitted.

Leahey, J.P., French, D.A., and Heath, J. (1985). PP321: Metabolism in a goat. ICI Plant Protection Division Report YIBH 85-C12, Sponsor Submitted.

McCormack, A. (1999a). Cyhalothrin: validation of an analytical method for determination in bovine edible tissues and milk. Report 808/115-D2140, Sponsor Submitted.

McCormack, A. (1999b). Cyhalothrin: validation of an analytical method for determination in ovine edible tissues. Report 808/116-D2140, Sponsor Submitted.

CYHALOTHRIN

First draft prepared by
Denis Hamilton, Queensland, Australia
Susan Calumpang, Manila, Philippines

Cyhalothrin was first evaluated by the 1984 JMPR when an ADI of 0-0.02 mg/kg bw was estimated. Cyhalothrin is a mixture in equal parts of four Z-*cis*-isomers, which exist in two enantiomeric pairs:

(R)-α-cyano (R)-*cis*-Z-cyclopropanecarboxylate and (S)-α-cyano (S)-*cis*-Z-cyclopropanecarboxylate, coded R157836

(S)-α-cyano (R)-*cis*-Z-cyclopropanecarboxylate and (R)-α-cyano (S)-*cis*-Z-cyclopropanecarboxylate, coded R119321 and PP321

Cyhalothrin was last evaluated by the JMPR in 1988. Cyhalothrin was originally developed for the control of livestock ectoparasites. The development for crop uses in countries other than Japan was changed from cyhalothrin to the enantiomeric pair coded PP321 (see above) because PP321 is more insecticidally effective. The spectrum of insecticidal activity is unchanged. Products containing this material are now available commercially in some 30 countries (JMPR,1986). Cyhalothrin has not been classified as fat-soluble or not fat-soluble. As cyhalothrin has a log P_{ow} = 6.8 (Pesticide Manual, 11th Edition) and the data on distribution between meat and fat as determined in the goat metabolism study, JMPR would likely classify cyhalothrin as fat-soluble. No animal commodity MRLs were set.

A system for assigning common names to synthetic pyrethroids and related compounds was still under discussion within ISO in 1986. Subject to the outcome of the discussion, it is expected that lambda-cyhalothrin will be proposed as a common name for PP321. For convenience, this name was used in the evaluation (JMPR, 1986)

USE PATTERN

Cyhalothrin is a pyrethroid insecticide with a high level of activity against a wide range of *Lepidoptera, Hemiptera, Diptera* and *Coleoptera* spp. and has miticidal activity. The compound is a stomach, contact and residual insecticide. It shows adulticidal, ovicidal and, particularly, larvicidal activity. It is also extremely effective against a number of insects resistant to standard treatments such as organochlorines and organophosphates. Like other photostable synthetic pyrethroids, cyhalothrin is relatively stable to degradation in sunlight. Cyhalothrin is not plant systemic and has very little fumigant or translaminar activity (JMPR 1984).

RESIDUES FROM SUPERVISED TRIALS

Wheat

Two residue trials were carried out on wheat during 1985 in The Netherlands. One application of cyhalothrin, 5% EC at 7.5 g ai/ha, was made either during or two weeks after flowering. No measurable residues (less than 0.01 mg/kg) were found in the mature grain at harvest (after 28/29 days) and only low residues, 0.06-0.18 mg/kg, in the straw (Kinkaid and Sapiets, 1986).

Cotton

Supervised trials in Brazil, Israel and South Africa involving repeated spray applications of a 2.5% EC formulation at rates of 6-20 g ai/ha, yielded residues at or below the LOD (0.01 mg/kg) in cotton seeds sampled 11-33 days after the last application (Sapiets, 1984a, b; Tyldesley and Sapiets, 1985). A program of four applications at rates of 50-150 g ai/ha, with a 21-day pre-harvest interval (PHI) yielded lambda cyhalothrin residue levels in cotton seed up to 0.18 mg/kg (Sapiets, 1984b). Multiple applications of lambda-cyhalothrin were conducted in the USA using a 12% EC formulation at 33-44 g ai/ha through commercial type spray equipment and a total of 13 sites distributed among nine US states country-wide. Lambda cyhalothrin was applied by air (4 sites); up to 15 applications were made, with the cotton harvested 20-27 days after last application. A lambda cyhalothrin residue of 0.01 mg/kg was detected in only one cotton seed sample, all other samples had no residues (LOD; 0.01 mg/kg) (Fitzpatrick, 1984; Neal, 1985a).

FATE OF RESIDUES

Plants

Cotton

^{14}C-cyclopropyl-labelled and ^{14}C-benzyl-labelled lambda-cyhalothrin (98% pure radio-chemically) were formulated as a 2.5% emulsifiable concentrate formulation, diluted with water and sprayed on cotton plants at a rate equivalent to 66 g ai/ha at flowering. These applications were repeated 3 and 7 weeks after flowering. The plants were grown to maturity. A residue of 0.010 mg cyhalothrin equivalents /kg was found in the cotton seeds from plants treated with ^{14}C-benzyl-labelled lambda-cyhalothrin. A residue of 0.020-0.027 mg cyhalothrin equivalents/kg was found in cotton seeds from plants treated with the cyclopropyl-labelled material. Although some characterization of the latter residue was possible, the levels of radioactivity present were too small to allow identification of any of the radioactive products present in these mature seeds (Leahey and French, 1985).

These data indicate that lambda-cyhalothrin and its metabolites are not readily translocated into cotton seeds following foliar application of lambda-cyhalothrin. The largest single source of residues on cotton seeds is likely to be from direct contamination of the seeds, by spraying after the bolls have begun to split.

To study the fate in the latter situation, ^{14}C-cyclopropyl-labelled and ^{14}C-benzyl-labelled lambda-cyhalothrin were formulated as a 2.5% emulsifiable concentrate formulation in water and spotted on to cotton seeds, in freshly ripened bolls, on plants maintained in a greenhouse for a further 14 days. 96% of the radioactivity present after 14 days was extractable with hexane; virtually all of it was present as lambda-cyhalothrin, with no significant change in isomeric composition (Leahey and French, 1985).

Growing cotton leaves were treated with the separated *cis* and *trans* isomers of ^{14}C-cyclopropyl-labelled [(RS)-α-cyano-3-phenoxy-benzyl-(1 RS)-*cis, trans*-3-(EZ-2-chloro-3, 3, 3-trifluoroprop-1-enyl)-2,2-dimethylclopropanecarboxylate] and were exposed to sunlight. After 28 days exposure, 68% (cis treatment) and 65% (trans treatment) of the radioactivity recovered from the leaves was due to unchanged pyrethroid. After 48 days, about 65% of the radioactivity on the *cis* compound treated leaves was polar, water-soluble compounds. On acid hydrolysis 66% was converted to organic soluble compounds, containing (1 RS)-*cis* and *trans*-3-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propanecarboxylic acid and two unknown compounds. Some photoisomerisation (less than 10%) at the 1, 3 bond of the cyclopropane ring of the pyrethroid occurred on the leaf surface after 28 days (Curl and Leahey, 1979).

Animals

Goat

A goat was dosed orally for seven days with ^{14}C-cyclopropyl-labelled lambda-cyhalothrin. The dose rate was equivalent to approximately 11 ppm. During dosing, the maximum residue level in the milk was 0.27 mg cyhalothrin equivalents/kg (mean value during days 3-7 of 0.21 mg/kg), virtually all of which was characterized as lambda-cyhalothrin. The update from JMPR 1986 is not clear on the expression of cyhalothrin residues – on a whole milk basis or a fat basis. The goat was slaughtered 16 hours after receiving the final dose, when residues in the tissues, expressed in cyhalothrin equivalents, were 0.024-0.028 mg/kg in meat; 0.13 -0.44 mg/kg in fat; 0.34-0.35 mg/kg in liver; and 0.20 mg/kg in kidney.

The residues in meat and fat were due mainly to lambda-cyhalothrin. However, in the liver and kidney, intact pyrethroid accounted for only a small part of the residue. (1RS)-*cis*-(Z-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylic acid and 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2-hydroxymethyl-2-methylcyclo-propanecarboxylic acid were the major components of the residue identified in liver and kidney (Leahey, French and Heath, 1985).

Cow

In the above-mentioned study with the goat, lambda-cyhalothrin was labelled in the acid portion of the molecule. This gave information on the fate of the whole molecule as well as of metabolites derived from the acid part. Information on metabolites derived from the alcohol portion of lambda-cyhalothrin was already available from previous studies conducted with cypermethrin, since the alcohol part of cypermethrin is identical to the alcohol part of cyhalothrin.

When ^{14}C-benzyl-labelled cypermethrin was administered orally to cows, the radioactive residues in the milk, fat and meat were identified as the parent compound. The only residues arising from ester cleavage and the alcohol half of cypermethrin were found in the liver and kidney, the major residue being N-(3-phenoxybenzoyl) glutamic acid.

Hydrolysis of the liver and kidney metabolites yielded 3-phenoxybenzoic acid and 3-(4-hydroxyphenoxy)benzoic acid (FAO/WHO, 1982).

Friesian cows were fed for up to 30 consecutive days on diets containing lambda-cyhalothrin at 1, 5 and 25 mg/kg. No effects were seen on milk yields or upon the general health of the animals. Lambda-cyhalothrin residues in milk correlated well with dietary inclusion rates, with mean plateau residue levels of 0.02 mg/kg, 0.09 mg/kg and 0.52 mg/kg, respectively for the 1, 5 and 25 mg/kg dietary inclusion rates. Lambda-cyhalothrin residue levels in milk did not accumulate and they declined when the feeding of the treated diet ceased. At the end of the 30 days, three cows from each group were slaughtered. The remaining two cows from the 25 mg/kg group were fed an untreated diet for a further 14 days before they were slaughtered. Lambda-cyhalothrin residue levels in the tissues were detected in the kidney, peritoneal and subcutaneous fat. Residues could not be detected in the liver while residues in the adduetes and pertoramuscle ranged from <0.01 to 0.05 mg/kg. (Sapiets, 1985). The update from JMPR 1986 is not clear on the expression of cyhalothrin residues – on a whole milk basis or a fat basis.

Processing Studies

Cotton

A study was conducted in the USA to determine the residues of PP321 (lambda-cyhalothrin) and its opposite enantiomer pair (R157836) in the processed fractions of cotton seed relative to the concentration in the whole ginned cotton seed. Cotton seed samples taken from a field trial in Goldsboro, North Carolina were processed to yield delinted cotton seed, hulls, linter motes, linters, lint, solvent extracted crude oil, refined oil and soapstock. In the field trial the cotton crop had received twelve applications of lambda-cyhalothrin at 33 g ai/ha. This was followed by three applications of an exaggerated rate of 100 g ai/ha, to ensure measurable residues. The insecticide was sprayed with ground equipment in spray volumes of 180 l/ha. The results showed that lambda-cyhalothrin was present in the ginned cotton seed as surface residues, most of which were removed in the delinting process. Concentrations of lambda-cyhalothrin in the delinted cotton seed, and in the process food fractions of the delinted cotton seed, were lower that those on the ginned cotton seed (Neal, 1985b).

METHODS OF ANALYSIS

The method of crop residue analysis for cyhalothrin is also applicable to lambda-cyhalothrin. It was reviewed by the 1984 meeting. The crop samples were prepared for analysis by mincing or chopping until homogenous. For small fruits the whole sample was prepared; large vegetables, e.g. cabbages, were quartered and opposite quarters taken. Samples were extracted using 50% acetone in hexane and the extracts washed with water. Co-extractives were removed by liquid-liquid partition chromatography where necessary (crops requiring this clean-up include cabbages). All crop extracts were cleaned up using Florisil adsorption chromatography. Final quantitative determination of cyhalothrin residues was by gas-liquid chromatography using electron-capture detection. The limit of determination for total cyhalothrin isomers is 0.01 mg/kg.

The analytical procedure used an internal standard, (SR)-α-cyano-3-phenoxybenzyl-(1RS)-*cis*-3-(Z-2-bromo-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate, for the quantitative measurement of residues. The internal standard was added to each sample prior to extraction. The response ratio of cyhalothrin to internal standard in the final extract of each sample was used for quantitative measurement of residues. Using the internal standard allows the size of the sample aliquot processed through the method to be reduced without loss of accuracy. Since the recovery of the pyrethroid is effectively monitored for each sample, the precision of the assay is significantly improved. Calibration curves plotted for cyhalothrin versus response ratio of cyhalothrin to the internal standard gave a linear regression (correlation coefficient r >0.99) with an intercept passing through the origin (Sapiets and Swaine, 1985).

MAXIMUM RESIDUE LIMITS

Codex MRLs have been established for cabbages, head, cotton seeds, cotton seed oil, crude, cotton seed oil, edible, pome fruits and potato. These are summarised in Table 1.

Table 1. Cyhalothrin MRLs as a pesticide

Main uses	8 INSECTICIDE
JMPR	84, 86R, 88R
ADI	0.02 mg/kg body weight (1984)
RESIDUE	Cyhalothrin (sum of all isomers)

Commodity Code	Name	MRL (mg/kg)	Step	JMPR	CCPR
VB 0041	Cabbages, head	0.2	CXL		
SO 0691	Cotton seed	0.02	CXL		
OC 0691	Cotton seed oil, Crude	0.02	CXL		
OR 0691	Cotton seed oil, Edible	0.02	CXL		
FP 0009	Pome fruits	0.2	CXL		
VR 0589	Potato	0.02	CXL		

The only crops in the Codex MRL table that could lead to residues in animals are cotton seed and pome fruits. The MRL for cotton seed is 0.02 mg/kg, so residues are very low or undetectable. The MRL for pome fruits is 0.2 mg/kg. No further information was provided on residues in crops leading to residues in animals even though the 1986 JMPR recognized the possibility (Desirable Further Information).

FURTHER WORK OR INFORMATION

The following information was requested by JMPR in 1986.

1. Further information on residues of cyhalothrin in foods of animal origin arising from feeding with treated crops or from direct treatments.

REFERENCES

Curl, E.A. and Leahey, J.P. (1979). "PP564: Degradation in Cotton Leaves", ICI Plant Prot. Div. Report. No. TMJ 1762B.

FAO/WHO, (1982). 1981 Evaluations: Pesticide Residues in Food.

Fitzpatrick, R.D. (1984). "PP321 Residues in cottonseed", ICI Americas Inc. Ag. Chem. Div. Report No. TMU 1518/B (unpublished).

Kimkaid, A. and Sapeits, A. (1986). Residue levels on wheat for 1985 trials in The Netherlands, ICI Report Series M 4198B.

Leahey, J.P. and French, D.A. (1985). "PP321: Characterization of radioactive residues found in cotton seeds from plants treated with ^{14}C-PP321 and degradation of ^{14}C-PP321 applied directly to cotton seeds". ICI Plant Prot. Div. Report No. RJ0393B

Leahey, J.P., French, D.A.and Heath, J. (1985). PP321: Metabolism in a goat, ICI Plant Prot. Div. Report. No. RJ0435B

Neal, J. (1985a). "PP321 residues in cottonseed", ICI Americas Inc. Ag. Chems Div. Report No. TMU 1778/B (unpublished).

Neal, J. (1985b). "PP321 residues in the process fractions of cottonseed", ICI Americas Inc. Ag. Chem. Div. Report. No. TMU 1805 (unpublished).

Sapiets, A. (1984a). Residue levels in cottonseed from a 1984 trial in Brazil, ICI Plant Prot. Div. Report No. M3826B

Sapiets, A. (1984b). Residue levels on cottonseed from a 1984 trial in Israel, ICI Plant Prot. Div. Report No. M3949B

Sapiets, A. (1985). PP321: Residue transfer study with dairy cows fed on a diet containing the insecticides, ICI Plant Prot. Div. Report. No. M3936B.

Sapiets, A and Swaine, H. (1985). The determination of residues of cyhalothrin in crops- a gas liquid chromatographic technology using an internal standard, ICI Plant Prot. Div. Residue Analytical Method (PPRAM) No. 70.

Tyldesley, D.J. and Sapiets, A. (1985). PP321: residue levels in cottonseed from a 1985 trial in South Africa, ICI Plant Prot. Div. Report No M4093B.

CYPERMETHRIN

First draft prepared by
Raymond Heitzman
Newbury, United Kingdom

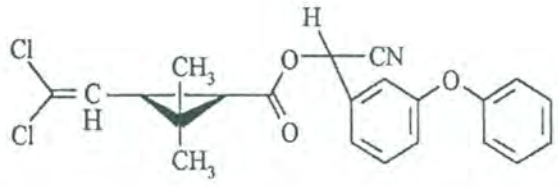
ADDENDUM
to the cypermethrin residue monograph prepared by the 47th meeting of the Committee and published in FAO Food and Nutrition Paper 41/9, Rome, 1997

IDENTITY

Chemical Structure: (RS)-α-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 (Chemical Abstracts name); CAS No. 52315-07-8

Cypermethrin is a mixture of all eight possible chiral isomers.

Structural Formula:



Molecular Formula: $C_{22}H_{19}Cl_2NO_3$
Molecular Weight: 416.3

Cypermethrin was first reviewed by the Joint Meeting on Pesticide Residues (JMPR) in 1979 and subsequently in 1981, 1986, 1988 and 1990. MRLs have been 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 on cattle, sheep and poultry by the 47th meeting of the Committee. Temporary MRLs were recommended for cattle, sheep and poultry in muscle, liver, kidney and fat. The Committee required the following information to further elaborate MRLs:

1. Radiodepletion 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 derived.

The Committee will need to ascertain the contribution of ingested pesticide residues from non-food animal sources and subtract this from the ADI to calculate the permitted theoretical maximum daily intake for cypermethrin from food animals when it is used as a veterinary drug.

PHARMACOKINETICS

One male and one female sheep were dosed orally with a mixture of 80% *cis* and 20% *trans* ^{14}C-cypermethrin isomers at 1mg/kg BW. Blood and excreta were collected over a seven-day period. Maximum plasma concentrations were observed at 8h for males (140 µg/kg) and 12h for females (144 µg/kg). The depletion half-life was 37h and 42h for the

male and female sheep, respectively. By 144h post-dosing, more than 95.8% of the radioactivity was cleared from the plasma. In males, 44% of the dose was cleared into the urine and most of the clearance was in the first 48h; in females, the corresponding value was 35%. In males, 30% of the dose was excreted into the faeces; in females, 35% of the dose was in the faeces.

Radiodepletion study in sheep

A new study was submitted investigating the radiodepletion of ^{14}C-cypermethrin administered orally to adult sheep, not topically as requested. Three groups of five sheep comprising at least two of each sex per group were dosed orally with 1mg/kg BW ^{14}C-cypermethrin. The ratio of the *cis* and *trans* isomers in the preparation was 4:1. The groups were slaughtered at 1, 3 and 5 days after dosing. Muscle, liver, kidney and fat samples were collected and the total residues were determined by combustion and liquid-scintillation counting. (see table 1).

Table 1. Total residues of cypermethrin in μg equiv./kg in sheep tissues after oral administration of 1mg/kg BW ^{14}C-cypermethrin.

Muscle	Individual values	Mean ± SD
Day 1	12, 10, 12, 17, 14	13 ± 2.7
Day 3	9, 8, 0, 10, 9	7.2 ± 4.1
Day 5	0, 0, 0, 0, 0	0 ± 0
Liver		
Day 1	348, 295, 331, 348, 349	334 ± 23
Day 3	99, 148, 145, 132, 151	135 ± 21
Day 5	59, 57, 69, 66, 77	66 ± 8
Kidney		
Day 1	425, 327, 416, 302, 569	408 ± 105
Day 3	56, 52, 87, 48, 57	60 ± 16
Day 5	18, 14, 18, 18, 18	17 ± 2
Fat		
Day 1	39, 40, 53, 70, 47	50 ± 13
Day 3	75, 90, 48, 75, 79	73 ± 15
Day 5	60, 46, 46, 49, 60	52 ± 7

The tissues collected from the five sheep slaughtered on day 1 post dosing were analysed for their content of both the *cis* and *trans* cypermethrin by radio-TLC. The ratio of marker residue to total residues in the edible tissues gave similar values to the results submitted for the 47th meeting by Crawford and Hutson (1977). The results are shown in table 2. No residues of the *trans* isomer were detected. This supports the observation in rats that the *trans* isomers are much more rapidly metabolised than the *cis* isomers (Casida et al., 1976).

Table 2. The total residues and marker residue content of sheep tissues 1 day after oral treatment with ^{14}C-cypermethrin at 1mg/kg BW.

Tissue.	Total Residues (μg equiv. /kg)	% TR extracted	*cis*-CYP (μg/kg)	*trans*-CYP (μg/kg)	Ratio CYP: TR	CYP: TR[1] (1980 data)
Muscle	13 ± 3	99.5 ± 24.5	3 ± 2	0	0.21	nm.
Liver	334 ± 23	71.7 ± 7.5	13 ± 5	0	0.04	<0.01
Kidney	408 ± 105	96 ± 2.7	5 ± 1	0	0.12	0.08
Fat	50 ± 13	107 ± 17	43 ± 16	0	0.86	0.65

Footnote: Data submitted to 47th meeting of the Committee.

APPRAISAL

No information has been submitted to answers requests 1 and 2 and there was no indication that the sponsors would provide this information in the near future.

A study in sheep treated orally with a radiolabelled 80:20 *cis:trans* isomer ratio was made available to the Committee. This study did not address the topical administration of cypermethrin, and the isomer ratio was different from that of the isomer mixture of 45:55 *cis:trans* cypermethrin which had been evaluated at the forty seventh meeting.

In answer to request 3, a suitable analytical method for measuring residues of the 80:20 *cis:trans* cypermethrin was submitted to the Committee. The method allows measurment of the Cypermethrin as the sum of all eight isomers. As the isomers were coeluted in the GC, the method could be used to measure the sum of the isomers in mixtures of Cypermethrin containing isomers at different ratios.

MRL

Since the Committee did not receive answers to its requests for further information, and there is no viable prospect that relevant information will be provided in the future, it is recommended that the temporary MRLs recommended for all animal tissues and milk are not extended. The Committee also noted that no information was made available for the toxicological evaluation of the 80:20 *cis:trans* cypermethrin.

REFERENCES

Beyerbach, A. (1999). ^{14}C-Cypermethrin : Absorption, distribution, metabolism and excretion in sheep. Draft report, Covance Laboratories for Vericore Ltd, Report No. 1412/021-D1141.

Casida, J. E., Ueda, K., Gaughan, L. C., Jao, L.T. and Sutherland, D.M. (1976), Structure-biodegradability relationships in pyrethroid insecticides. Arch. Environ. Contam. Tox., 3, 491-500.

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

CYPERMETHRIN

First draft prepared by
Denis Hamilton, Queensland, Australia
Susan Calumpang, Manila, Philippines

Cypermethrin was reviewed by the JMPR in 1979 and 1981-86, 1988 and 1990; MRLs have been recommended on a wide range of crops, meat and milk products and feed commodities.

Cypermethrin is active against a wide range of insects, which attack crops and can be used at a relatively low dose rate in the rage of 0.02-0.25 kg ai/ha. It is a moderately stable and water insoluble compound. The technical material contains not less than 90% w/w cypermethrin, which is a mixture of eight optical isomers, with a cis: trans isomer ratio of approximately 40:60. The maximum concentrations of residues in/on the treated crops are in the range of 0.05 – 2 mg/kg and decreases slowly (JMPR, 1979).

USE PATTERN

Animals

In Australia, three products containing cypermethrin are registered for ectoparasite control. "Barricade s" cattle dip and spray (25 g/l cypermethrin and 138 g/l chlorfenvinphos) is used at 19-21 day intervals to control cattle ticks *Boophilus microplus*. Dipping is used in *exiqua* infestation for up to 21 days. It controls other ticks (*Haematobia irritans* and *Ixodes holocyclus*), cattle lice *Linognathus vituli, Damalinia bovis* and *Hematopinus eaurysternus,* and buffalo fly on horses, deer, goats and sheep. It is diluted 1:250 with water giving a cypermethrin concentration of 100 mg/l. Outflank off-shears pour-on sheep lice treatment, contains 25 g/l cypermethrin. The application rate is 10 ml along the back line of shorn sheep weighing up to 50 kg, and 15 ml for sheep over 50 kg. One application controls sheep lice (*Damalinia ovis*) and ked (*Melophagus ovinus*). Control is achieved in 4-6 weeks. Outflank plunge and shower sheep dip, containing 47.5 g/l cypermethrin, diluted 1:2500, giving a cypermethrin concentration of 19 mg/l, is used for the control of lice and keds on sheep.

RESIDUES FROM SUPERVISED TRIALS

Plants

Wheat

Residue trials have been carried out on wheat in Canada and Brazil, with application rates up to 150 g/ha. In the grain residues did not exceed 0.1 mg/kg at harvest two weeks after application. Residues in the straw were higher, and the few data obtained suggest that under these conditions they may approach 10 mg/kg. The 1979 report from JMPR is not clear on the rate applied - 150 g ai/ha or 150 g formulation/ha.

Animals

Cow

Cattle were treated with 25 g/L cypermethrin formulation as an overspray treatment to control buffalo flies. Cattle were sprayed along the dorsal midline at concentrations of 0.1 and 0.2% ai (200 ml per animal). At 1, 3, 8 and 15 days post treatment, three animals were slaughtered and various samples collected. Residues in muscle, liver, and kidney at both dosage rates were all below the limit of determination (0.01 mg/kg) in animals treated once only. In animals treated twice, determinable residues ranged from 0.01 to 0.05 mg/kg (Shell, 1979). It is not clear if the residues are expressed on fat tissue or other tissue.

Other trials were conducted on dairy cows using a cypermethrin/chlorfenvinphos acaricidal mixture by plunge dipping in a bath containing 0.075 g/l of cypermethrin. Samples were taken from the bulked milk of a commercial herd at intervals up to 14 days and from six individual cows at intervals to seven days, after treatment. Three of the individual cows were dipped again on the eighth day with samples taken six days later. Residues of cypermethrin between <0.002 and 0.01 mg/kg were found in samples taken up to three days after treatment; declining to below the limit of determination (0.002 mg/kg) at the end of the first week. This report from 1986 is not clear on the expressions of cypermethrin residues- on a whole milk basis or a fat basis. However, concentrations expressed per kg (litre) suggest the whole milk.

Cattle were also dipped once or twice in a bath containing 0.075 g/l of cypermethrin using an interval of 7 days. Animals were slaughtered 1, 3, 4 or 7 days after the first dip and some animals at 7 days after a second dip. Residues in liver, kidney and muscle were all below the limit of determination (0.01 mg/kg) while residues in omental fat ranged from <0.01-0.02 mg/kg and in perirenal fat at 0.01-0.05 mg/kg.

Sheep

Animals (24 wethers) were manually dunked into a dip containing 0.01% cypermethrin. Residues were detected in omental fat, perirenal fat and muscle ranging for <0.01 (0 day) to a high of 0.17 mg/kg (in perirenal fat at 14 days after 3 dippings). Residues could not be detected in liver and kidney.

A 1982 Australian trial was conducted using a 2.5% cypermethrin pour-on sheep dip applied to merino ewes at 15 ml (normal maximum dosage rate) and 30 ml. The application was on a line 15-25 cm along the back line between shoulder and anterior pelvis. Sheep were slaughtered 1, 3, 7, 14 and 28 days after treatment and samples of muscle (neck), kidney, liver, omental fat and perirenal fat were analysed for residues of cypermethrin. For the recommended maximum dose rate of 15 ml of 2.5% cypermethrin, residues in both ometnal and perirenal fats reached peak values of 0.04 mg/kg after 7 days. Values for a double dose rate of 30 ml also peaked after 7 days at 0.07 mg/kg for omental fat and 0.08 for perirenal fat. The results for muscle, kidney and liver samples were all less than 0.02 mg/kg.

Goat

Four milking goats were dunked in a dipping vat containing 0.01% cypermethrin three times after the 0, 4 and 8-day morning milkings. Samples of homogenized goat milk were examined. Residues were detected in goat milk after 18 days (mean 0.002 mg/kg).

FATE OF RESIDUES

Plants

Residue trials have been carried out on wheat in Canada and Brazil, with application rates up to 150 g/ha. In the grain residues did not exceed 0.1 mg/kg at harvest two weeks after application. Residues in straw were higher and the few data obtained suggest that under these conditions they may approach 10 mg/kg.

Following applications to various crops cypermethrin may degrade to hydrolysis and oxidation products. The most likely degradation products present in crops at harvest following normal agricultural use of cypermethrin are the derived amide (compound B), 3-phenoxybenzoic acid and 2-(2′, 2′-dichloro vinyl)-3,3-dimethyl cyclopropane carboxylic acid (compound C). The latter two compounds are found in the free state as well as in conjugated forms. However, the evidence indicates that the major component of any residue present at harvest will be cypermethrin. Several crop samples obtained from supervised trials were analyzed also to determine residues of compounds B, C and phenoxybenzoic acid. The results of these examinations involving some 20 crops showed no residues of compound B, C or of 3-phenoxy benzoic acid in excess of 0.05 mg/kg.

Animals

Cattle

Two radiolabelled studies were undertaken to investigate cypermethrin residues in cattle and whether residues in meat or milk could arise from its use in feed containing products made from treated crops.

Low Dietary Intake (0.2 mg/kg)

Two lactating cows were given feed concentrate containing radiolabelled cypermethrin twice daily for a 3-week period at a feeding level equivalent to 0.2 mg/kg on total daily feed. The initial radioactivity in milk (time frames not noted) amounted to only 0.5% of radioactivity fed to animals. About 60-70% of the radioactivity in the milk was present in the cream fraction. The remainder was excreted in the urine (54%) and faeces (43%). At the end of the feeding period, cypermethrin residues were below 0.001 mg/kg in blood, muscle and brain. In subcutaneous and renal fats, liver and kidney samples, residue were at or below 0.012 mg/kg cypermethrin equivalents.

High Dietary Intake (5 mg/kg)

Radiolabelled cypermethrin was given in the feed concentrate twice daily at an amount equivalent to 4 mg/kg on total diet over a period of 7 days. It is not clear from the 1979 JMPR report what actual concentrations were used (5 mg/kg or 4 mg/kg).

The major excretory route was via the kidneys. The cream fraction contained 85-90% of the total radioactivity in the milk samples. These results indicate that cypermethrin does not accumulate in the animal tissues. Even at high intake levels, residues were mostly in the fat, liver and kidney. Cereals and components of feed (e.g. cotton seed) were treated with the highest recommended dose rates. The majority of residue in the feed is excreted in the urine and faeces. Therefore, measureable residues are unlikely to result in meat or milk of cattle. The residues in the milk are esters and contain both acidic and alcoholic moieties of the parent compound. The main metabolite in urine is 3- phenoxybenzoic acid as the glutamic and glycine derivatives. The faeces mainly contain the parent molecule.

Poultry and poultry products

Both radio- and non-labelled studies on the nature and level of residues in eggs and poultry meat were reviewed. Cypermethrin levels in whole eggs reached a plateau after approximately seven days of 0.05-0.09 mg/kg at the 40 ppm dietary level (the highest tested) with the residues being mainly in the yolk. Levels declined rapidly when dosing was stopped. The highest poultry flesh levels were 0.06 mg/kg at the 40 ppm dose level. It is unlikely that residues of parent compound or metabolites will reach significant levels in eggs or poultry meat, since dietary levels will seldom exceed 1 ppm in the poultry whole diet for any appreciable period. The JMPR proposed an MRL of 0.05 mg/kg for eggs and poultry meat.

Processing

Cypermethrin is a moderately stable and water insoluble compound. Data relating to the effect on residue levels of various treatments given to harvested crops are as follows:

Crop peelings

Numerous data show that the residue in a crop is largely on the surface. Analyses of pulp and peel after peeling apples, pears, peaches and citrus fruits show that levels in the pulp were below 30% of the applied dose, and in most instances below 10%, of those in whole fruit.

Oil seeds

Cottonseed was deliberately treated at the high rate of 300 g/ha and harvested one day after treatment. Cottonseed contained 0.12 mg/kg cypermethrin on a whole seed basis, with adhering linters. The sample was processed by simulating commercial practices in a laboratory specializing in the technique. Residues were transferred to kernels that originally did not contain any detectable residue in the seed during the commercial mechanical separation process. The cypermethrin residues in the extracted oil at various stages are: crude oil 0.10 mg/kg, neutralized oil 0.07 mg/kg, bleached oil 0.08 mg/kg and deodorized oil 0.05 mg/kg. Both the alkali wash and deodorization steps contribute to some losses. The results from this experiment suggest that the two processes together may be expected to remove about half of the residue. Hence, it is possible that residues may occasionally occur in oil obtained from seed treated under practical conditions at levels approaching those in whole seed. The report from JMPR (1979) is not clear on the rate applied on the cotton seed sample - 300 g ai/ha or 300 g formulation/ha.

METHODS OF ANALYSIS

Several methods have been developed for residue analysis of agricultural commodities. The methods are all based on gas-liquid chromatography procedures using equipment commonly found in modern analytical laboratories. Limits of determination of 0.01–0.02 mg/kg are usually attainable.

Experiments have shown that most samples may be stored for long periods in the deep freeze without appreciable loss of residues. Residue levels were determined in crop and soil samples at intervals following addition of known quantities of cypermethrin at 0.2-1 mg/kg. The samples were stored at –18°C from treatment to analysis, for 1-54 weeks for crops and 4-49 weeks for soil. Recoveries of cypermethrin added to crops (21 samples) were 85-110%, apart from one sample of tobacco that reported yields of 45% after 6 months storage. In the case of soils (5 samples) recoveries were all 90-110% (Shell R. 152).

Studies have also been carried out on the stability of residues of 3-phenoxybenzoic acid and Compounds B and C in storage at –18°C. Over three months there was no evidence of loss of 3-phenoxybenzoic acid, since the total amounts recovered from the 6 crops used (lettuce, potatoes, cabbage, apples, pears and maize grain) were 75-100% and comparable to recovery values of the method itself. With Compound B experiments were carried over three months with sweet corn and over five months with apples and cabbage. Recoveries were 70%, 75% and 75%, respectively. Cabbage and apples treated with Compound C were also stored at –180°C for five months. Recoveries were 90-95%, respectively. These data indicate that loss of any of the three degradation compounds mentioned were negligible over periods of 3-5 months at –18°C.

APPRAISAL

Degradation in crops occurs mainly by hydrolysis of the ester bond followed by further hydrolytic and oxidative processes to give a variety of products. Less rapid processes observed were hydrolysis of the nitrile group to amide and hydroxylation of the phenoxy-ring. The compounds formed were, in turn, also hydrolyzed at the ester link. However, metabolites have not been detected in crop commodities from supervised trials. At least 90% of the total residue present in plant material is extractable with acetone. Processing of treated crops after harvest usually reduces the residue significantly (JMPR 1979).

Cypermethrin is readily absorbed, distributed and metabolized in mammals. Because of the chemical and especially the isomeric complexity of the molecule, the metabolic profile with respect to all of its isomers is extremely complex. Cypermethrin is readily cleaved at the ester linkage and subjected to oxidative degradation and conjugation of the metabolic products. Elimination following acute and subacute administration is rapid. However, the clearance rate from adipose tissue is slow and a half-life in rats and mice may range from 10-30 days. The data suggest a potential for bioaccumulation following continuous exposure (JMPR 1979).

Cattle consuming feed items treated with cypermethrin eliminate the residue rapidly. Equilibrium between intake and excretion is reached in 3-4 days. The total radioactivity found in milk was only 0.5% of the radioactivity fed to the animals; 60-90% of this residue is present in the cream fraction. The residues in the milk are esters and contain both acidic and alcoholic moieties of the parent compound. The majority of the residue in feed is excreted in the urine and faeces in similar proportions. The main metabolite in urine is 3-phenoxybenzoic acid present as glutamic acid conjugate and glycine derivatives. The faeces mainly contain the intact molecule.

Cypermethrin does not accumulate in muscle tissue. Even when fed at high dose rate, residues are mostly in the fat, liver and kidney. The data indicate that the feeding of crops treated with cypermethrin following the recommended use patterns does not result in measurable residues in meat or milk of cattle. Since the compound may be used for direct treatments of animals, and data deriving from the latter use are not available, recommendations of MRLs for animal products were not made at the 1979 JMPR meeting.

From the information on residues resulting from the use of cypermethrin for ectoparasite control and feeding studies, it was concluded that residues of cypermethrin are not likely to exceed 0.2 mg/kg for meat (in the fat) and 0.05 mg/kg for muscle, liver, kidney and milk.

MAXIMUM RESIDUE LIMITS

Codex MRLs have been established for a variety of fruits and vegetables. MRLs for animal products include: edible offal (mammalian), eggs, meat (from mammals other than marine mammals), milks and poultry meat. Feed commodities with MRLs include barley, maize, maize fodder, sorghum straw and fodder, dry, soya bean (dry), wheat and wheat straw and fodder, dry. MRLs are summarized in Table 1.

Table 1. Cypermethrin residues in crops and meat products

Main uses	8 INSECTICIDE
JMPR	79, 81, 82R, 83R, 84R, 85 R, 86 R, 88R, 90R (00R')
ADI	0.05 mg/kg body weight (1981; confirmed in 1996 by JECFA)
RESIDUE	Cypermethrin (sum of isomers) (fat-soluble)

Code	Commodity	MRL (mg/g)	Step	JMPR	CCPR
AL 1021	Alfalfa forage (green)	5 dry wt	CXL		
GC 0640	Barley	0.5	CXL		
VP 0062	Beans, shelled	0.05	CXL		
FB 0018	Berries & other small fruits	0.5	CXL		(1991)
VB 0040	Brassica vegetables	1	CXL		
FS 0013	Cherries	1	CXL		
FC 0001	Citrus fruits	2	CXL		
SB 0716	Coffee beans	0.05	CXL		
VP 0526	Common bean (pods and/or Immature seeds)	0.5	CXL		
VC 0424	Cucumber	0.2	CXL		
MO 0105	Edible offal (mammalian)	0.05 V	CXL		
VO 0440	Egg plant	0.2	CXL		
PE 0112	Eggs	0.05	CXL		
VL 0480	Kale	1	CXL		
VA 0384	Leek	0.5	CXL		
VL 0482	Lettuce, Head	2	CXL		
GC 0645	Maize	0.05	CXL		
AS 1645	Maize fodder	5 dry wt	CXL		
MM 0095	Meat (from mammals other than marine mammals	0.2 (fat) V	CXL		
ML 0106	Milks	0.05 (fat) V	CXL		
VO 0450	Mushrooms	0.05	CXL		
FS 0245	Nectarine	2	CXL		
SO 0089	Oilseed, except peanut	0.2	CXL		
VA 0385	Onion, bulb	0.1	CXL		
FS 0247	Peach	2	CXL		
SO 0697	Peanut	0.05	CXL		
VP 0063	Peas (pods and succulent-immature seeds)	0.05	CXL		
VO 0051	Peppers	0.5	CXL		
FS 0014	Plums (including prunes)	1	CXL		
FP 0009	Pome fruits	2	CXL		
PM 0110	Poultry meat	0.05	CXL		
VR 0075	Root and tuber vegetables	0.05	CXL		
AS 0651	Sorghum straw and fodder, Dry	5	CXL		
VD 0541	Soya bean (dry)	0.05	CXL		
VL 0502	Spinach	2	CXL		
VO 0447	Sweet corn (corn-on-the-cob)	0.05	CXL		
DT 114	Tea, Green, Black	20	CXL		
VO 0448	Tomato	0.5	CXL		
OR 0172	Vegetable oils, Edible	0.5	CXL		
GC 0654	Wheat	0.2	CXL		
AS 0654	Wheat straw and fodder, Dry				

FURTHER WORK OR INFORMATION

Desirable

1. Use pattern for animal health use and residues in animal products deriving from the recommended application (JMPR 1979)

REFERENCES

Australia, (1980), Cypermethrin residues in animal tissues and milk. Submission to the 1986 JMPR.

Shell Chemical (Australia) Pty. Ltd. (1979). Cypermethrin residue data in animal tissues and milk. Project S/AU/D 2-78 (unpublished).

DICYCLANIL

First draft prepared by
Stefan Soback, Beit Dagan, Israel
Rainer Stephany, Bilthoven, The Netherlands
Robert Wells, New South Wales, Australia

IDENTITY

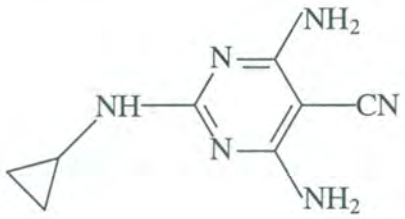
Chemical name: 4,6- diamino-2-(cyclopropylamino)-5-pyrimidinecarbonitrile
Chemical Abstract Service (CAS): CAS number:112636-83-6

4,6-diamino-2-cyclopropylaminopyrimidine-5-carbonitrile
International Union of Pure and Applied Chemistry (IUPAC) name:

International Non-Proprietary Name (INN): DICYCLANIL

Synonyms: Clik, A-9568 B, CGA 183893

Structural formula:



Molecular formula: $C_8H_{10}N_6$
Molecular weight: 190.2

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Dicyclanil
Appearance: White crystalline powder.
Melting point: 250.5-252.4 °C with thermal decomposition
Thermal stability: >150 °C
Solubility: 4900 mg/L in methanol, 1400 mg/L in ethyl acetate, 1200 mg/L in acetone, 320 mg/L in octanol, 610 mg/L in buffer solution at pH 5.0 and 350 mg/L water at pH 7.0. Poorly soluble in hexane, toluene and dichloromethane.
Dissociation constant: pKa = 4.58 (basic)
Ultraviolet maxima: Not reported.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use
Dicyclanil is an ectoparasiticide of the aminopyrimidine group used for the control of myiasis and fly-strike in sheep. Dicyclanil is sold as a pour-on formulation containing 5% (w/v) of the compound. Data were provided for use in sheep only.

Dosage

Dicyclanil is applied as a single, seasonal spray-on treatment using an applicator gun provided with the drug. The recommended use is approximately 1-2 ml of the formulated product (5g dicyclanil/100 ml) per kg body weight according to the following guide:

	Body weight of the sheep	
(kg)	ml	ml/kg
10-20	20	2.0-1.0
21-30	25	1.2-0.8
31-50	30	1.0-0.6
>50	35	0.7

According to these instructions the maximal administered amount of the active compound is 1.75 g/animal while the maximal dose is 0.1 g dicyclanil/kg body weight.

METABOLISM

Laboratory animals

Rats

Dicyclanil metabolism was investigated in 2 studies in which ^{14}C-labeled dicyclanil (5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine) was administered orally to rats (Hassler, 1994) and (Thanei, 1996). The [2-^{14}C]pyrimidyl labeled CGA 183893 was given to the rats orally by a stomach tube at two different doses (20 mg/kg and 0.5 mg/kg) for 7 consecutive days. The rats weighed 200 g at the initiation of the study. Four study groups were used consisting of 6 animals (3 males and 3 females) each. More than 80% of the administered dose was absorbed and 24 hours after the last of the 7 doses, radioactivity was recorded in all tissues. The radioactivity was recovered mainly in urine and feces representing 79-85% and 6-13%, respectively. Consequently, 24 hours after the last of the 7 administrations 93-96% of the total radioactivity was recovered. By use of thin layer TLC) and high performance liquid chromatography (HPLC) a total of 12 metabolite fractions were separated from urine, feces and tissues. No sex or dose dependency was observed. One of the urinary fractions represented 50% of the given dose and was the major metabolic product of CGA 183893.

In the second study (Thanei, 1996) identification of the metabolites was attempted. Approximately 20 mg/kg was given to the test animals and samples were obtained from 4 rats (2 male and 2 female). Urine samples were collected from 4 rats over a period of 168 h and pooled for analysis. Fecal samples from the 2 male rats and the 2 female rats were also pooled for analysis. The rat liver tissue 24 hours after the last dose were obtained for analysis. For the identification of the metabolites, ^{1}H-NMR, IR and mass spectrometry were used. The following compounds were characterized:
N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide (MET 1U),
5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine (MET 2U = CGA 183893),
2-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-3-hydroxy-propionic acid (MET 3U),
2-4,6-triamino-pyrimidine-5-carbonitrile (MET 4U = CGA 297107),
3-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-propionic acid (MET 5U)

Biotransformation was initiated by oxidative cyclopropyl-ring opening followed by further oxidation. Biotransformation was limited to the cyclopropyl-ring while the cyano group is metabolically stable. The mode of action of compounds containing cyclopropyl group has been studied at length, indicating interaction with P450 enzymes as a possible mode of action. The reaction mechanism most frequently involves cyclopropyl ring opening resulting in free radicals. Inhibition of redox reactions of enzyme systems has been associated to these groups. The most significant route was the conversion of the CGA 183893 to MET U1 consisting of 50% of the administered dose.

Food producing animals

Sheep

In a study by Phillips (1996), specimens from male and female sheep, treated with a pour-on formulation containing radiolabeled [^{14}C-pyrimidine] dicyclanil, from an earlier study were used. The urine, feces, bile, wool, fat, muscle, liver, and kidney samples were pooled. Thin layer chromatography (TLC) was used for characterization of the metabolites.

One and two-dimensional TLC were performed. The portion of the dose remaining in the wool was investigated at 3 and 21 days after treatment. The wool contained mainly unchanged parent compound (CGA 183893) but low levels of CGA 297107 and an unknown component were also detected.

In urine, collected for 48 hours after administration and representing <1% of the total dose, CGA 183893 and CGA 297107 accounted for 63 and 6% of the urine radioactivity, respectively. Six additional unidentified components were also detected of which 3 accounted for <1% of radioactivity and the 3 others for 10%, 7% and 1%. Less than 1% of the total radioactivity was detected in the pooled feces from samples collected during 48 hours post-administration. Exhaustive extraction recovered 91% of the fecal radioactivity. Unchanged CGA 183893 accounted for 72% and CGA 297107 for 2% of the combined extracts. The residuals consisted of polar material and unresolved radioactivity. The bile contained polar components and unresolved components.

Extractable radiolabelled compounds from fat and muscle tissue was high (over 90%), containing mainly the parent compound in the subcutaneous fat and the parent compound and unresolved matter in the peritoneal fat and muscle. The extractability from liver was 20-31% and subsequent soxhlet extraction released an additional 17-24%. The TLC analysis of the extractable substance was inconclusive due to the extremely low levels of radioactivity. From kidney 58-72% was extracted with additional 14-20% following soxhlet extraction. As with the liver, the TLC analysis was inconclusive due to low radioactivity present.

The extractable radiolabelled residues were high at all time points from muscle and fat while the extractability from liver and kidney was less. The major residue in fat and muscle tissues was the parent compound (CGA 183893). In liver and kidney most of the extractable residues were characterized as unresolved CGA183893 and an unknown compound. CGA 297107 was identified in a kidney extract.

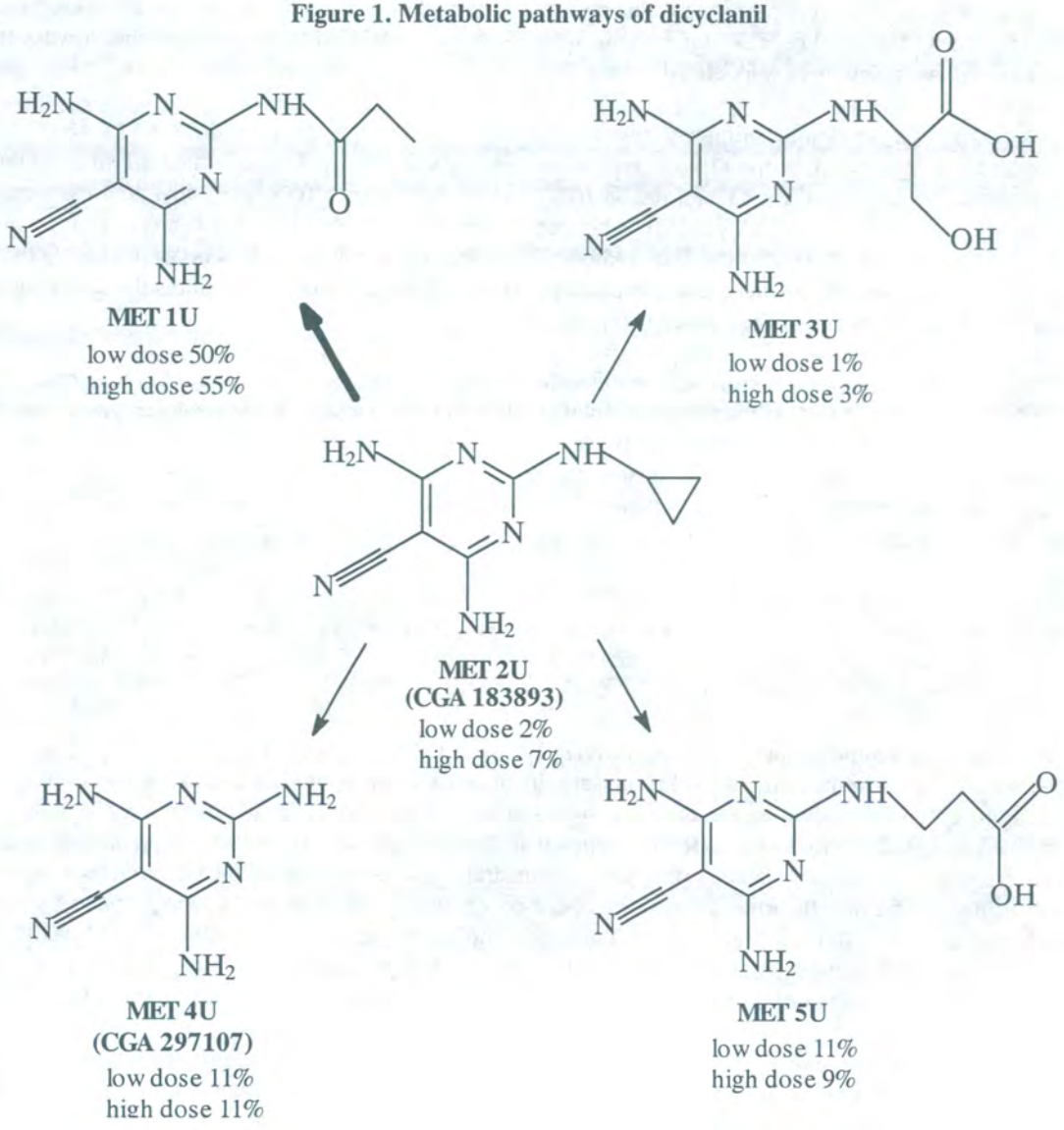
Thanei (1996a) studied the metabolic pattern of dicyclanil in sheep utilizing TLC and HPLC techniques. Also in this study, urine, feces, bile, dose run-off, wool, fat, muscle, liver and kidney samples obtained from another study were utilized. About 40-60% of the administered dose was found in the run-off during the first hour after administration. The run-off contained essentially only CGA 183893 except for traces of CGA 287107. The same substances in the same proportions were found in the wool 1 and 14 days post-administration. Less than 2% of the dose retained on the skin was recovered in urine and faeces. In urine 5 fractions could be distinguished: N-(4,6-diamino-5-cyano-pyrimidin-2-yl)-propionamide (MET 1U), 5-cyano-2-cyclopropylamino-pyrimidin-4,6-diamine (MET 2U = CGA 183893), 2-4,6-triamino-pyrimidine-5-carbonitrile (MET 4U = CGA 297107), 3-(4,6-diamino-5-cyano-pyrimidin-2-ylamino)-propionic acid (MET 5U) and a polar unidentified fraction that released CGA 297107 following microwave treatment.

The extractability of radiolabelled products was about 90% from feces and consisted almost entirely of CGA 183893. In bile the majority of radioactivity appeared to be associated with very polar metabolites. However, unchanged CGA 183893 and CGA 297197 were also found.

Extractability of the radioactivity from adipose and muscle tissue was almost 100% and the major component in these tissues was the unchanged CGA 183893. CGA 287107 was present in low amounts in these tissues as well as the MET 1U in muscle tissue. In kidney and liver tissues, extractability decreased as a function of time. In the kidney the initial extractability was 90% but decreased to 50% in 14 days. In the liver 40-60% of the radioactivity was extractable and an additional 20% could be extracted under harsh conditions. The extractable metabolites had a half-life of about 1 day. The concentration of non-extractable residues was 0.006 and 0.002 mg/kg in kidney and liver, respectively.

The metabolic pathways in sheep were essentially the same as found in rats. The metabolic conversion going through cyclopropyl-ring opening and oxidation of the α-carbon to a secondary propionic acid amide (MET 1U) or cyclopropyl-ring opening and oxidation to a β-alanine-derivative (MET 5U) and dealkylation to descyclopropyl CGA 183893 (MET 4U = CGA 297107). The proposed dicyclanil metabolic pathways are presented in Figure 1.

Figure 1. Metabolic pathways of dicyclanil



TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies in sheep

Three sheep studies were reported with target animals using a radiolabeled dicyclanil formulation typical of those used in current veterinary applications (Gifford and Dunsire, 1994, Thanei, 1996a and Anderson and Speirs, 1998).

In one study (Gifford and Dunsire, 1994) the ^{14}C-dicyclanil was administered by use of the jetting technique (the other studies used a pour-on formulation). With the jetting technique, 35 mg dicyclanil/kg was applied as a single dose to Oxford Down sheep. Approximately 37-59% of the total dose remained on the animals while the rest could be recovered as "run-off". Dermal absorption was estimated as 2% based on the radioactivity retained on the animal. The absorption was rapid and the maximum whole blood concentration appeared 4-6 hours after the drug administration. During 7 days following the drug administration, 0.83% and 1.05% of the retained dose was recovered in urine and feces, respectively. The ^{14}C-label compounds recovered in the urine were the parent compound and 4 metabolites of which each fraction consisted of less than 0.2%. The fecal excretion was predominantly the parent compound. Considerable radioactivity could be recovered from the wool. The radioactivity in the wool did not decrease notably with time (Gifford and Dunsire, 1994 and Thanei 1996a)

The second study was carried out by administering the ^{14}C-dicyclanil as a pour-on to Grayface sheep at 35 mg dicyclanil/kg (MacLean and Dunsire, 1996). In this study the maximum blood concentration was reached at 12-48 hours and the absorption was slower than in the previous study. The absorption of the administered dose over a period of 7

days was 4%, twice as much after the pour-on administration when compared to the administration by jetting. This study also indicated continuous dermal absorption, something not observed in the previous study. In this study the radioactivity was recovered in urine and feces. The metabolic pattern generally agreed with the previous study although the ratio of the metabolites differed from the earlier studies (McLean and Dunsire, 1996 and Phillips, 1996).

The third study was carried out by administering the ^{14}C-dicyclanil as a pour-on to Dorset sheep at 100 mg dicyclanil/kg (Anderson and Speirs, 1998). Moderate run-off was noticed. Radioactivity was detected in the systemic circulation at 2 hours and the peak concentrations at 24 after administration. The absorption over 21 days was about 7% of the administered dose. Biphasic excretion of the radioactivity was observed. The decline of radioactivity could be characterized by a half-life of approximately 2 days during the period of 24-120 hours post-administration. Radioactivity was widely distributed to the body and the highest radioactivity count was recorded in the subcutaneous fat under the application area. From day 7 to day 21 the radioactivity decreased most in blood and muscle and least in omental and subcutaneous fat.

Other residue depletion studies in sheep

All together eight studies were performed in sheep. Three of these studies were GLP non-compliant and the remaining five studies were done in accordance to the GLP standards. Different parameters were investigated including formulation, dose, application method and wool length. Furthermore, the studies were not identical in terms of compounds (parent and metabolites) analyzed.

Dicyclanil was applied by jetting the formulation in 3 liters per animal (1.5g) at 29 to 44 mg/kg body weight onto 8 Merino sheep with wool length of 2-3 cm (Strong and Kearney, 1992). Tissue from two animals was collected at 2, 7, 14 and 28 days after application. Muscle, fat, liver and kidney tissues were analyzed for the presence of the parent compound. No residues exceeding 10 µg/kg were detected in muscle, liver or kidney tissues (20 µg/kg in kidney). A dicyclanil residue of 20 µg/kg was found in one fat tissue sample at 7 days and another at 14 days post application. Deposits of 1200-2500 mg/kg of dicyclanil were measured in the wool. Results are tabulated in Table 1.

Table 1. Dicyclanil residues (mg/kg) in sheep tissues after 1.5 g topical administration.

Withdrawal time (days)	Muscle	Liver	Kidney	Fat
2	<0.01	<0.01	<0.02	<0.01
7	<0.01	<0.01	<0.02	<0.02
14	<0.01	<0.01	<0.02	<0.02
28	<0.01	<0.01	<0.02	<0.01

The second study involved 15 Merino wethers, shorn 2 weeks before treatment (Bull, 1995). The sheep were divided to 5 groups of 3 animals each. The sheep (mean ±SD) in the groups were from 55 ±6 to 60 ±7 kg. A 2.5-3 times the label dose of 90 mg/kg body weight was given as a topical application of 1.8 ml/kg of a pour-on formulation. Animals per time point were sacrificed at 3, 7, 14 and 21 days after application. Three untreated control animals were sacrificed before the first group of treated animals. Hind leg muscle, kidney fat, liver and kidney tissue samples were analyzed for the presence of the parent compound. The highest residue concentrations were found in muscle (0.93 mg/kg), in liver and in kidney (1.4 mg/kg) 7 days after the treatment; in fat tissue, 1.89 mg/kg was observed at 3 days after treatment. The dicyclanil residues decreased to ≤0.30 mg/kg by day 14 but apparently increased again by day 21 (Table 2). No explanation was provided for the day 14 versus day 21 results.

Table 2. Mean dicyclanil residues (mg/kg) in sheep after 90 mg/kg topical administration.

Withdrawal time (days)	Muscle	Liver	Kidney	Fat
3	0.75	0.98	0.66	0.92
7	0.71	0.90	0.82	0.36
14	0.12	0.13	0.18	0.18
21	0.58	0.68	0.57	0.20

The third study involved 23 Merino wethers, shorn 2 weeks before treatment (Smal and Adams, 1995). The animals were divided to 6 groups of 3 sheep. The mean body weight in the groups ranged from 53.0 to 54.0 kg. The two

sheep with the lowest body weight (mean = 39.4kg) were allocated as the untreated control animals. A dose of 45 mg/kg body weight was given as a topical application of 0.9 ml/kg of a pour-on formulation. Three animals per time point were sacrificed at 3, 7, 14, 21, 28, 35 and 42 days after application. Three control animals were sacrificed before the first group of the treated animals. Hind leg muscle, kidney fat, liver and kidney tissue samples were analyzed for dicyclanil . The highest residue concentrations were found in muscle (0.16 mg/kg), in liver (0.33 mg/kg) and in kidney (0.21 mg/kg) 21 days after the treatment. The highest concentration in fat tissue (0.67 mg/kg) was also observed at 21 days after treatment. The residues of dicyclanil decreased to <0.0.1 mg/kg by day 42 after treatment in muscle and liver tissue (Table 3).

Table 3. Mean dicyclanil residues (mg/kg) in sheep with 45 mg/kg topical administration.

Withdrawal time (days)	Muscle	Liver	Kidney	Fat
7	0.09	0.14	0.09	0.17
14	0.06	0.08	0.06	0.26
21	0.10	0.14	0.12	0.64
28	0.09	0.10	0.08	0.05
35	0.04	<0.03	<0.04	0.09
42	<0.01	<0.01	<0.04	0.07

In the forth study 78 lambs (2nd cross: 25% Merino, 25% Border Leicester and 50% Dorset), containing equal number of wethers and ewes, were used (Smal *et al.*, 1996). The animals were ranked by sex and weight and divided to light, medium and heavy groups. From each sub-group, animals were randomly assigned to 2 treatment groups of 3 animals to include one lamb from each weight group. The mean body weight in the groups ranged from 40.7 to 41.7 kg. The control sheep, containing 3 males and 3 females, had a mean body weight of 44.2 kg. A dose of 2 ml/kg or 4 ml/kg, was given as a topical pour-on formulation using an applicator gun. Three animals per time point from each treatment group were sacrificed at 3, 7, 14, 21, 28 and 35 days after application. Three control animals were sacrificed before the first slaughter of the treated animals. Equal mix of tenderloin and hindquarter muscle, kidney and associated fat, liver and subcutaneous fat (from the application area) were analyzed for the parent compound and the major metabolite. Results are tabulated in Table 4a-4d. The highest residue concentrations of parent and metabolite, respectively, were: in muscle (0.18 and 0.08 mg/kg), liver (0.29 and 0.26 mg/kg), kidney (0.25 and 0.37 mg/kg) and adjoining (0.03 and <0.01 mg/kg) and subcutaneous (0.05 and 0.01 mg/kg) fat samples 14 days after the treatment with the lower dose. The highest residue concentrations, after administration of the higher dose, of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.17 and 0.09 mg/kg), liver (0.28 and 0.24 mg/kg), kidney (0.26 and 0.20 mg/kg) and adjoining (0.04 and 0.02 mg/kg) and subcutaneous (0.06 and <0.01 mg/kg) fat 3 days after the treatment. The highest concentration of the major metabolite in muscle tissue (0.10 mg/kg) was found at 21 days after treatment.

Table 4a. Mean dicyclanil concentrations (mg/kg) in sheep after topical administration at 45 mg/kg.

Withdrawal time, (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.01	<0.01	<0.01	<0.01	<0.01
7	<0.01	<0.02	<0.02	<0.01	<0.01
14	<0.04	<0.06	<0.05	<0.02	<0.01
21	<0.01	<0.01	<0.01	<0.01	<0.01
28	<0.01	<0.01	<0.01	<0.01	<0.01
35	<0.01	<0.01	<0.01	<0.01	<0.01

Table 4b. Mean dicyclanil concentrations (mg/kg) in sheep after topical administration at 90 mg/kg.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.05	<0.07	<0.07	<0.03	<0.02
7	<0.01	<0.02	<0.02	<0.01	<0.01
14	<0.02	<0.01	<0.01	<0.01	<0.01
21	<0.03	<0.03	<0.03	<0.01	<0.01
28	<0.03	<0.03	<0.03	<0.01	<0.01
35	<0.01	<0.02	<0.02	<0.01	<0.01

Table 4c. Mean CGA 287107 concentrations in sheep (mg/kg) after dicyclanil topical administration at 45 mg/kg.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.02	0.04	0.04	<0.01	<0.01
7	<0.02	0.05	0.06	<0.01	<0.01
14	<0.03	0.07	0.11	<0.01	<0.01
21	<0.01	0.03	0.04	<0.01	<0.01
28	<0.01	<0.03	0.04	<0.01	<0.01
35	<0.01	<0.02	<0.02	<0.01	<0.01

Table 4d. Mean CGA 287107 concentrations in sheep (mg/kg) after topical administration of dicyclanil at 90 mg/kg.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
3	<0.04	0.12	0.10	<0.01	<0.01
7	0.03	0.08	0.08	<0.01	<0.01
14	0.02	0.05	0.06	<0.01	<0.01
21	0.04	0.07	0.07	<0.01	<0.01
28	<0.02	0.08	0.08	<0.01	<0.01
35	<0.01	0.05	0.05	<0.01	<0.01

The fifth study used 43 Merino wethers and 41 Merino ewes (Peterson and George, 1997). The animals were assigned by weight to 4 groups. Two groups were treated immediately after shearing with normal and high doses and 2 other groups were dosed six weeks after shearing using similar treatments. Five slaughter groups for each of the 4 treatment groups and consisting of 2 males and 2 females each were generated. The mean body weight was 43-47 kg, 45-46 kg, 51-54 kg and 53-55 kg in the four treatment groups, respectively. A dose of 2 ml/kg and 4 ml/kg, was given as a topical application over the backline as a pour-on formulation using an applicator gun. Three animals per time point from each treatment group were sacrificed at 7, 14, 21, 28 and 58 days post treatment. Four untreated control animals representing the 3 heaviest males and the heaviest female were sacrificed before the first slaughter of the treated animals. Tenderloin and hindquarter muscle, kidney, renal fat, liver and subcutaneous fat (from the application area) were analyzed for the presence of the parent compound and the major metabolite. Results are summarized in Tables 5a-5h.

In the group receiving the lower dose immediately after shearing the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.76 and 0.19 mg/kg), liver (1.13 and 0.36 mg/kg), kidney (0.20 and 0.50 mg/kg), renal fat (0.13 and 0.03) and subcutaneous fat (0.28 and 0.06 mg/kg) samples 7 days after the treatment except for diclycnil residues in subcutaneous fat and the metabolite residues in renal fat at 14 days post-administration.

In the group receiving twice the lower dose immediately after shearing, the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (1.18 and 0.56 mg/kg), liver (1.83 and 2.59 mg/kg), kidney (1.58 and 0.63 mg/kg), renal fat (0.20 and 0.06) and subcutaneous fat (3.29 and 0.07 mg/kg) samples 7 days after the treatment except for the diclycnil residues in subcutaneous fat in a sample obtained 14 days post-administration.

In the group receiving the lower dose 6 weeks after shearing, the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.32 and 0.13 mg/kg), liver (0.45 and 0.24 mg/kg), kidney (0.36 and 0.30 mg/kg), renal fat (0.08 and 0.01) and subcutaneous fat (0.62 and 0.02 mg/kg) samples 14 days after the treatment except for the metabolite residues in muscle and kidney at 7 days post-administration and in the subcutaneous fat in a sample obtained 28 days post-administration.

In the group receiving the higher dose 6 weeks after shearing, the highest residue concentrations of both analyzed compounds (parent and metabolite, respectively) were found in muscle (0.95 and 0.41 mg/kg), liver (1.38 and 0.68 mg/kg), kidney (1.22 and 0.98 mg/kg), renal fat (0.11 and 0.07) and subcutaneous fat (3.86 and 0.08 mg/kg) in samples

7 days after the treatment except for the metabolite residues in kidney, in renal fat and in the subcutaneous fat measured in samples at 14 days post-administration.

Table 5a. Mean dicyclanil concentrations (mg/kg) in sheep treated with a 2 ml/kg topical dose immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.32	0.42	0.35	0.08	0.04
14	0.12	0.12	0.08	0.10	0.02
21	0.03	0.04	0.02	0.01	0.01
28	0.02	0.02	0.01	0.01	<0.01
56	0.05	0.08	0.04	0.04	0.01

Table 5b. Mean dicyclanil concentrations (mg/kg) in sheep treated with a 4 ml/kg topical dose immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous Fat	Renal fat
7	0.80	1.21	0.94	0.24	0.16
14	0.34	0.46	0.33	0.89	0.06
21	0.20	0.32	0.22	0.05	0.03
28	0.14	0.22	0.18	0.04	0.03
56	0.02	0.02	0.02	0.02	<0.01

Table 5c. Mean dicyclanil concentrations (mg/kg) in sheep treated with a 2 ml/kg topical dose at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous Fat	Renal fat
7	0.18	0.24	0.20	0.04	0.02
14	0.13	0.18	0.14	0.21	0.03
21	0.05	0.07	0.05	0.03	0.01
28	0.05	0.05	0.04	0.12	0.01
56	0.02	0.02	0.04	<0.01	<0.01

Table 5d. Mean dicyclanil concentrations in sheep (mg/kg) treated with a 4 ml/kg topical dose at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.58	0.81	0.73	0.20	0.08
14	0.40	0.59	0.43	1.46	0.09
21	0.24	0.39	0.33	0.08	0.05
28	0.18	0.22	0.16	0.03	0.03
56	0.10	0.20	0.02	0.03	0.02

Table 5e. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 2 ml/kg topical dose of dicyclanil immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.12	0.24	0.39	0.03	0.01
14	0.07	0.14	0.34	0.02	0.01
21	0.04	0.11	0.11	0.01	0.01
28	0.02	0.08	0.10	0.01	<0.01
56	0.03	0.06	0.06	0.01	<0.01

Table 5f. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 4 ml/kg topical dose of dicyclanil immediately after shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.48	0.49	0.41	0.05	0.04
14	0.11	0.23	0.24	0.03	0.01
21	0.12	0.37	0.34	0.03	0.01
28	0.10	0.18	0.26	0.02	0.01
56	0.03	0.08	0.07	<0.01	<0.01

Table 5g. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 2 ml/kg topical dose of dicyclanil at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.10	0.15	0.23	0.01	<0.01
14	0.07	0.15	0.16	0.01	<0.01
21	0.04	0.09	0.13	0.01	0.01
28	0.03	0.08	0.08	0.01	<0.01
56	0.01	0.03	0.05	<0.01	<0.01

Table 5h. Mean CGA 287197 concentrations in sheep (mg/kg) treated with a 4 ml/kg dose of dicyclanil at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Subcutaneous fat	Renal fat
7	0.25	0.44	0.46	0.03	0.01
14	0.20	0.37	0.48	0.04	0.03
21	0.08	0.28	0.30	0.02	0.01
28	0.08	0.20	0.14	0.01	0.01
56	0.03	0.09	0.06	0.01	0.01

In the sixth study, 20 Merino and 20 2nd cross lambs were used (Peterson and George, 1997a). The two lightest and heaviest animals of both breeds were selected as the untreated control group. The remaining animals were randomly assigned to 3 slaughter groups to contain 6 animals of both breeds. The mean body weight of the groups was about 35 kg. A dose of 2 ml/kg was given as a topical application 6 weeks after shearing over the backline as the "Clik" formulation using an applicator gun. Six animals per time point from each group were sacrificed at 11, 28 and 35 days after application. The four control animals were sacrificed before the first slaughter of the treated animals. Tenderloin muscle, liver, kidney and renal fat were analyzed for the presence of the parent compound and the major metabolite.

In the Merino group, the highest residue concentrations of parent and metabolite, respectively, were found in muscle (0.10 and 0.09 mg/kg), liver (0.11 and 0.10 mg/kg), kidney (0.14 and 0.28 mg/kg) and renal fat (0.03 and 0.02) in samples 11 days after the treatment. In the cross-breed group, the highest residue concentrations of parent and metabolite, respectively, were found in muscle (0.04 and 0.05 mg/kg), liver (0.07 and 0.11 mg/kg), kidney (0.06 and 0.11 mg/kg) and renal fat (0.03 and 0.02) samples 11 days after the treatment. Results are summarized in Tables 6a-d.

Table 6a. Mean dicyclanil concentrations (mg/kg) in cross breed lambs treated with a 2 ml/kg topical dose at 6 weeks off shears.

Withdrawal times (days)	Muscle	Liver	Kidney	Renal fat
11	0.01	0.02	0.02	0.01
28	<0.01	<0.01	<0.01	0.01
35	<0.01	<0.01	0.01	<0.01

Table 6b. Mean dicyclanil concentrations (mg/kg) in merino sheep treated with a 2 ml/kg topical dose at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Renal fat
11	0.03	0.04	0.04	0.01
28	<0.01	0.01	<0.01	0.01
35	<0.01	<0.01	0.01	<0.01

Table 6c. Mean CGA 287197 concentrations (mg/kg) in cross breed lambs with a 2 ml/kg topical dose of dicyclanil at 6 weeks off shears.

Days after	Muscle	Liver	Kidney	Renal fat
11	0.03	0.07	0.08	<0.01
28	<0.01	0.03	0.03	<0.01
35	<0.01	0.03	0.04	<0.01

Table 6d. Mean CGA 287197 concentrations (mg/kg) in merino sheep with a 2 ml/kg topical dose of dicyclanil at 6 weeks off shears.

Withdrawal time (days)	Muscle	Liver	Kidney	Renal fat
11	0.06	0.07	0.19	0.01
28	0.02	0.04	0.06	<0.01
35	0.01	0.03	0.07	<0.01

The seventh study contained 22 Merino sheep and 22 second cross lambs of both sexes aged 14 months to 6 years (Smal and George, 1997). The mean body weight of the Merino group was 42 kg and 26 kg in the cross breed group. The lightest and heaviest animal of both breeds was selected as the untreated control group. The remaining animals were randomly assigned to 5 slaughter groups with 4 animals of both breeds. The Merinos were given a topical application of 1 ml/kg and the cross-breeds 2 ml/kg using the "Clik" formulation over the backline with an applicator gun 1 day after shearing. Six animals per time point from both groups were sacrificed at 7, 28, 56, 84 days and 4 months after application. The four control animals were sacrificed before the first group of treated animals. Tenderloin muscle, liver, kidney, subcutaneous fat from the application area and renal fat were analyzed for the parent compound and the major metabolite.

In the Merino group, the highest residue concentrations of parent and metabolite found, respectively, were in muscle (0.02 and 0.03 mg/kg), in liver (0.03 and 0.05 mg/kg), in kidney (0.03 and 0.06 mg/kg), in renal fat (<0.01 and <0.01 mg/kg) and in subcutaneous fat (0.03 and <0.01 mg/kg) samples 56 days after treatment except for the subcutaneous fat sample where the highest residues were obtained at 84 days post-treatment. In the cross-breed group, the highest residue concentrations of parent and metabolite, respectively, were found in muscle (<0.01 and 0.01 mg/kg), liver (<0.01 and 0.03 mg/kg), kidney (0.02 and 0.04 mg/kg), renal fat (0.03 and 0.01 mg/kg) and subcutaneous fat (0.13 and 0.04 mg/kg) samples obtained 7 days post-treatment except for the kidney and renal samples which were obtained 28 days after treatment.

The eight study (Hotz, 1999) was performed in order to determine the effect of wool length at the time of treatment on the residues in tissues. Fifty-two White alp sheep of both sexes were used. The mean weight of the males and females, respectively, were 43 kg and 36 kg. The animals were divided to two groups to be treated 1 day off shears and 7 weeks after shearing. The sheep were given dicyclanil at 2 ml/kg using an applicator gun. Tissue samples were collected 7, 14, 21, and 35 days after dosing. Six animals were killed at each sampling time from both treatment groups. Two male control animals from both groups were sacrificed before dicyclanil administration. Dicyclanil and CGA 297107 concentrations were determined in hindquarter, forequarter and tenderloin muscle, omental, renal, subcutaneous and subcutaneous application site fat, liver and kidney tissues. At 7 days post administration the concentrations of dicyclanil in muscle tissue ranged from 0.02 to 0.19 mg/kg and CGA 297107 from <0.01 to 0.17 mg/kg. At 14 and 21 day post-administration neither concentration exceeded 0.06 mg/kg (except for 0.33 mg/kg CGA 297107 in one sample). At 35 days after administration the highest CGA 297107 concentration was 0.02 mg/kg while the dicyclanil concentration was below the limit quantification in all but 2 (0.01 mg/kg) of 36 samples. The highest dicyclanil concentration was found in the omental and subcutaneous fat. The highest concentration was 0.97 mg/kg at 7 days and 0.19 at 35 days after

administration. The CGA 297107 concentrations in fat were at or below the limit of quantification. The highest dicyclanil concentration in liver was 0.30 mg/kg and 0.18 mg/kg in the kidney 7 days after administration while the respective CGA 297107 concentrations were 0.50 and 0.48 mg/kg. Dicyclanil and CGA 297107 concentrations in liver and kidney declined rapidly and were at or below the limit of detection at 35 days post administration.

METHODS OF ANALYSIS

The analytical approach for determination of dicyclanil residues in muscle, liver, kidney and fat tissues is based on high performance liquid chromatography (HPLC). The developed method allows separation of dicyclanil (CGA 183893) from its metabolite (CGA 297107). The limit of quantification (LOQ) for both compounds was 0.01 mg/kg. Two sample clean-up procedures were described. The extraction procedures were coded 239A and 239A.01. Both methods use aqueous acetonitrile for the primary extraction followed by filtration and separation of the lipids by a C18 solid phase cartridge. Additional clean-up was achieved by Tox Elut® solid phase cartridges. Final cleanup was performed by use of a quaternary methyl amine (method 239A) or a strong anion exchange (method 238A.01). The CGA 183893 and CGA 297107 were eluted separately in different fractions.

The separation in the HPLC was obtained by use of a strong cation exchange column. The mobile phase consisted of acetonitrile:0.01M sodium perchlorate:perchloric acid (70:30:0.1). The CGA 183893 and CGA 297107 eluted at different times. In the method 239A, using C18 μBondapak column and acetonitrile:water (20:80), only CGA 183893 elutes. The use of dual amine column and acetonitrile:water (99.5:0.5) elutes only CGA 297107. The LOQ of the two last methods was 0.02 and 0.1 mg/kg, respectively.

The method 239A.01 has been fully validated according to the requirements of the European Union for setting maximum residue limits for medicinal products.

APPRAISAL

Dicyclanil has not previously been reviewed by the Committee. Data were provided on the use of dicyclanil applied as a pour-on to sheep only. Most of the studies were conducted according to current GLP standards. Dicyclanil metabolism was well characterised and residue depletion was studied extensively taking into account application technique, dose, wool length, sex, race and age differences. In considering all the residue depletion studies, the mean ratio of marker residue to total residue for liver is (MR/TR) 0.15 in liver and 0.25 in kidney. Under almost all conditions the amount of adsorption of drug was low; the preferred treatment being a pour-on application. Most studies were conducted using higher than the recommended label doses.

Two analytical methods were described which allowed separate detection of dicyclanil and its CGA 297107 metabolite. The methods had a limit of quantification of 0.01 mg/kg.

MAXIMUM RESIDUE LIMITS

The following factors were considered in recommending MRLs:

1. An ADI of 0 - 0.007 mg/kg of body weight based on a toxicological endpoint was established, permitting a maximum daily intake of 0.42 mg for a 60 kg person.

2. The appropriate marker residue is the parent dicyclanil.

3. Due to the limited extractability of dicyclanil residues from the liver and kidney tissue (about 50%) as well as the ratio between dicyclanil and its main metabolite in tissues (30:70 in liver and 50:50 in kidney), a correction factor expressed as MR/TR (marker residue per total residue) was applied. The ratio (correlation factor) for liver was 0.15 and for kidney 0.25.

4. Dicyclanil residues can be detected using liquid chromatography (HPLC) based methods with a limit of quantification of 0.01 mg/kg. The method is appropriate to meet regulatory needs. No confirmatory method was provided in the submitted information.

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

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues in sheep, expressed as parent drug: muscle, 0.2 mg/kg; liver, 0.4 mg/kg; kidney, 0.4 mg/kg; and fat, 0.15 mg/kg

Based on consumption of 300 g of muscle, 100 g of liver, 50 g of kidney and 50 g of fat and using the factors for liver and kidney as given above, the theoretical maximum daily intake of dicyclanil residues from veterinary use is 0.42 mg/kg.

Table 7. Estimate of theoretical maximum daily intake of dicyclanil

Food Item	MRL (mg/kg)	Food Basket (kg)	mg	MR/TR[1]	TMDI (mg)
Muscle	0.2	0.3	0.06	1	0.06
Liver	0.4	0.1	0.04	0.15	0.27
Kidney	0.4	0.05	0.02	0.25	0.08
Fat	0.15	0.05	0.0075	1	0.01
Total:					**0.42**

[1] MR = marker residue (parent drug); TR = total residues

REFERENCES

Anderson, A.R. and Speirs, G.C. (1998). The absorption, distribution and excretion of [Pyrimidine-2-^{14}C] CGA 183893 after pour-on administration to sheep. Report No. 14072. Inveresk Research International, Trenent, Scotland.

Bull, M.S. (1995). Preliminary residue studies with CGA 183893 in sheep following application of CGA 183893 Pour-on. Report No. 95/5/1491. Yarrandoo Research Center, Ciba-Geigy, Australia Ltd. Kemps Creek, Australia.

Gifford, L.J. and Dunsire, J.P. (1994). Absorption, distribution and excretion of [2-^{14}C]Pyrimidyl CGA 183893 after topical administration to sheep. Report No. 10067. Inveresk Research International, Trenent, Scotland.

Hassler, S. (1994). Absorption, distribution and elimination on [2-^{14}C]Pyrimidyl CGA 183893 in the rat after multiple oral administration. Report 7/94. Division Crop Protection, Ciba-Geigy Limited, Switzerland.

Hotz, R.P. (1999). Residues of CGA 183893 in tissues, blood and plasma after pour-on administration to sheep with different wool length. Report CRA 99/017. Noartis Centre de Recherche Sante Animale SA, St-Aubin, Switzerland.

MacLean, C.L. and Dunsire, J.P. (1996). Absorption,distribution and excretion of [Pyrimidine-2-^{14}C] CGA 183893 after pour-on administration to sheep. Report No. 13372. Inveresk Research International, Trenent, Scotland.

Peterson, S.M. and George B. (1997). Dissipation of dicyclanil and its metabolite CGA 297107 in tissues of merino sheep following application at 0 and 6 weeks off-shears of dicyclanil as a Pour-on. Report No. 97/4/1559. Novartis Animal Health, Australasia Pty. Ltd. Kemps Creek, Australia.

Peterson, S.M. and George B. (1997a). Dissipation of dicyclanil and its metabolite CGA 297107 in tissues of merino sheep following application of dicyclanil. Report No. 97/8/1576. Novartis Animal Health, Australasia Pty. Ltd. Kemps Creek, Australia.

Phillips, M. (1996). [Pyrimidine-2-^{14}C] CGA 183893: Nature of metabolitesin the excreta and tissues of sheep following single pour-on administration. Report No. 14072. Inveresk Research International, Trenent, Scotland.

Smal, M.A., Adams, S. and Ochudzawa, Z. (1996). Dissipation of dicyclanil and its metabolite CGA 297107 in the tissues of fattening lambs following application of dicyclanil as a pour-on. Report No. 96/2/1517. Yarrandoo Research Center, Ciba-Geigy, Australia Ltd. Kemps Creek, Australia.

Smal, M.A. and George, B. (1997). Depletion of residues of dicyclanil and its metabolite CGA 297107 in tissues of lambs and adult merino sheep following application of dicyclanil as a spray-on. Report No. 97/12/1591. Yarrandoo Research Center, Novartis Animal Health, Australasia Pty. Ltd. Kemps Creek, Australia.

Smal, M.A. and Adams, S. (1995). Dissipation of CGA 183893 in the tissues of sheep following application of CGA 183893 as a Pour-on. Report No. 95/8/1496. Yarrandoo Research Center, Ciba-Geigy, Australia Ltd. Kemps Creek, Australia.

Strong, M.B. and Kearney, E.M. (1992). Preliminary residue studies with CGA 183983 in sheep following application by jetting. Report No. 92/5/1357. Yarrandoo Research Center, Ciba-Geigy, Australia Ltd. Kemps Creek, Australia.

Thanei, P. (1996). The metabolism of [2-^{14}C]Pyrimidyl CGA 183893 in the rat. Report 7/96. Division Crop Protection, Ciba-Geigy Limited, Switzerland.

Thanei, P. (1996a). The nature of metabolism in tissues and excreta of sheep after single topical administration of [2-^{14}C]Pyrimidyl CGA 183893. Report 9/96. Division Crop Protection, Ciba-Geigy Limited, Switzerland.

FLUMEQUINE

First draft prepared by
Robert Wells, NSW, Australia

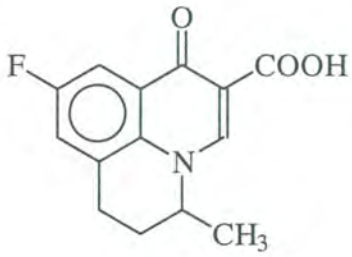
ADDENDUM
to the flumequine residue monograph
prepared by the 48th meeting of the Committee and published in
FAO Food and Nutrition Paper 41/10, Rome 1998.

INDENTITY

Chemical names: 9-Fluoro-6,7-dihydro-5-methyl-1-oxo-*1H,5H*-benzo[ij]-quinolizine-2-carboxylic acid.

Synonyms: R-802, Apurone.

Structural formula:



Molecular formula: $C_{14}H_{12}NO_3$

Molecular weight: 261.26

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: flumequine.

Appearance: white microcrystalline powder.

Melting point: 253-255°C.

Solubility: soluble in aqueous alkaline solutions and alcohol, insoluble in water.

Optical rotation: produced and used as a racemic mixture.

INTRODUCTION

The Committee has previously considered the antimicrobial agent flumequine at its forty-eighth meeting. At that meeting the Committee recommended MRLs for flumequine in cattle of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat, expressed as parent drug. In the absence of data on the contribution of parent drug to the total residues in sheep, chickens and pigs, the Committee recommended temporary MRLs in these species of 500 µg/kg for muscle, 1000 µg/kg for liver, 3000 µg/kg for kidney and 1000 µg/kg for fat, expressed as parent drug. The Committee also recommended a temporary MRL of 500µg/kg for trout muscle (including normal proportions of skin) expressed as parent drug.

In reaching its decision on MRLs for flumequine, the forty-eighth meeting of the Committee took the following factors into consideration:

- An ADI of 0-30 μg /kg of body weight was established, based on a toxicological end-point. This corresponds to a maximum theoretical daily intake of 1800 μg for a 60-kg person.
- The parent drug was identified as the marker residue.
- Muscle and kidney were considered to be the appropriate target tissues. For practical reasons, however, liver is recommended as the target tissue for chickens in place of kidney.
- On the basis of data from studies in calves, non-extractable residues accounted for 20% of the total residues.
- In calf muscle, kidney and fat, the parent drug accounted for approximately 80% of the extractable residues.
- 7-Hydroxyflumequine and the unknown metabolite, M1, together account for 80% of the total radioactivity in calf liver after 168 hours.
- On the basis of the contribution of parent drug in extractable residues in calves, the parent drug accounts for 50% of the total residues in muscle, kidney and fat, and 25% of the total residues in liver.
- No data were provided for milk or eggs and no MRLs are recommended.
- No data were provided on the amount of parent drug as a percentage of the total residues in sheep, chickens, pigs or trout.

From the MRLs for cattle, the maximum theoretical intake of flumequine residues would be 1100 μg per day, which is compatible with a maximum of 1800 μg for a 60-kg person based upon the ADI of 0-30 μg/kg body weight.

The Committee requested that studies be conducted with radiolabeled flumequine in pigs, sheep, chickens and trout to estimate the proportion of the total residues accounted for by the parent drug. The results of these studies were required for evaluation in 2000.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of Use

Flumequine is a first generation drug of the fluoroquinolone group of antibiotics. It is used primarily for the treatment of enteric infections in domestic species. Flumequine also has a limited use in man for the treatment of urinary tract infections.

METABOLISM

Previous studies of the metabolism of flumequine in calf liver

Metabolites were isolated from calf liver at different times after the last drug administration by extraction with both ethyl acetate and methanol. The efficiency of recovery of radioactivity was not significantly affected by enzymatic deconjugation for early time points (6 and 24h) but an increasing fraction of radioactivity was recovered in the ethyl acetate layer after deconjugation with *Helix promatia* as time increased. About 20% of the isolated metabolite radioactivity could be ascribed to conjugated metabolites. Acceptable recoveries of the residual radioactivity remaining in tissue after solvent extraction could be achieved using pronase digestion prior to repetition of the extraction process.

Radio profiling of peaks obtained from HPLC identified 13 other metabolites (M1-M13) in addition to flumequine and 7-hydroxyflumequine. Only flumequine and 7-hydroxyflumequine and metabolite M1 contributed significantly to total extractable radioactivity and M1 was the major metabolite identified at times of 24 h and later. Table 1 shows results of metabolite distribution after metabolite deconjugation with *Helix promatia.* The concentration of flumequine decreased rapidly and was not observable after 24 h. 7-Hydoxyflumequine decreased somewhat more slowly during the first 72 h (FAO, 1998).

Table 1. **Time dependence of the 3 major metabolites of flumequine in calf liver following hydrolysis with *Helix promatia***

Time(h) post dosing	Mean total radioactivity (mg equiv/kg)	Mean (%) total radioactivity measured	Analytes (% of total)		
			M1	(1)	(2)
6	7.73	65.5	9-15	42-58	9-13
24	5.41	51.0	25-41	10-34	2-12
72	4.19	48.2	26-43	ND	6-9
120	3.98	41.4	52-57	ND	ND
168	3.00	48.5	35-63	ND	0-22

Note: (1) = flumequine, (2) = 7-hydroxyflumequine. ND = not detected.

New metabolism studies with [^{14}C]-flumequine

Cattle

Three male and three female beef cattle, seven months old and weighing 125-135 kg, were given ^{14}C-flumequine for five consecutive days at a dose of 12 mg/kg body weight by subcutaneous injection into the neck. The first 3 of 4 doses were given in the left side of the neck and the last dose was injected into the right side of the neck. All animals were killed and exsanguinated 18 h after the final injection. The entire kidney, liver and longissimus dorsi muscle, fat and injection site were retained for HPLC analysis, determination of total radioactivity and microbiological activity. The fat used in these determinations was a composite of the renal, omental and subcutaneous fat of each animal.

The results of HPLC and radiochemical analysis are summarised in Table 2, showing that at the time of sacrifice about 83% of the radioactivity in liver is flumequine, either as drug metabolites or bound residues. Metabolites or bound residues account for about 21% of the radioactivity in both muscle and kidney while in fat and at the injection site, flumequine was recovered almost entirely as parent drug (Guyonnet, 1999; Lynch and McLean, 1998)

Table 2. **Ratio of flumequine to total radioactivity in cattle tissues after the s.c. administration of ^{14}C-labelled flumequine for 5 consecutive days**

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle	321	89	374	92	0.79 (0.04)
Liver	1200	280	7110	1380	0.17 (0.06)
Kidney	4200	981	5320	1270	0.79 (0.05)
Fat*	1080	666	1180	954	1.00 (0.14)
Injection site	455000	456000	464000	434000	1.04 (0.07)

Note: * fat samples were a composite of renal, omental and subcutaneous fat.

Pigs

Three male and three female pigs, weighing 45-49 kg were given ^{14}C-flumequine intramuscularly into the neck twice daily for five consecutive days. The first dose was 15 mg/kg body weight and the subsequent nine doses were 7.5 mg/kg body weight. Injections 1-9 were given in the left side of the neck and the last dose was injected into the right side of the neck. All animals were killed and exsanguinated 16 h after the final injection. The entire kidney, liver and longissimus dorsi muscle, fat and injection site were retained for HPLC analysis, determination of total radioactivity and microbiological activity. The fat used in these determinations was a composite of the renal, omental and subcutaneous fat of each animal.

The results of HPLC and radiochemical analysis are summarised in Table 3, showing that at the time of sacrifice about 93% of the radioactivity in liver is flumequine, either as drug metabolites or bound residues. Metabolites or bound residues account for about 56% of the radioactivity in kidney, 45% in skin with fat and about 25% in both muscle and fat. (Guyonnet, 1998a; Lynch and Speirs, 1998a)

Table 3. Ratio of flumequine to total radioactivity in pig tissues after i.m. administration of ^{14}C-labelled flumequine twice-daily for 5 consecutive days

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle	199	45	262	42	0.75 (0.05)
Liver	468	78	7140	964	0.07 (0.02)
Kidney	2360	861	5270	796	0.44 (0.10)
Fat*	364	113	483	141	0.76(0.09)
Skin with fat	246	41	446	59	0.55 (0.05)
Injection site	104000	57600	125000	68000	0.83 (0.20)

Note: * fat samples were a composite of total fat isolated.

Sheep

Three male and three female sheep, weighing 38-50 kg, were dosed twice daily with ^{14}C-flumequine intramuscularly into the neck for five consecutive days. The first dose was 12 mg/kg body weight and the subsequent nine doses were 6 mg/kg body weight. Injections 1-9 were given in the left side of the neck and the last dose was injected into the right side of the neck. All animals were killed and exsanguinated 16 h after the final injection. The entire kidney, liver and longissimus dorsi muscle, fat and injection site were retained for HPLC analysis, determination of total radioactivity and microbiological activity. The fat used in these determinations was a composite of the renal, omental and subcutaneous fat of each animal.

The results of HPLC and radiochemical analysis are summarised in Table 4, showing that at the time of sacrifice about 94% of the radioactivity in liver is flumequine, either as drug metabolites or bound residues. Metabolites or bound residues account for about 65% of the radioactivity in kidney, 51% in muscle and 44% in fat. At the left and right injection sites, lower observed levels of metabolites were accompanied by high amounts of flumequine (Guyonnet, 1998b; Lynch and Speirs, 1998b).

Table 4. Ratio of flumequine to total radioactivity in sheep tissues after i.v. administration of ^{14}C-labelled flumequine twice-daily for 5 consecutive days

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle	49	15	100	21	0.49(0.08)
Liver	139	30	2520	787	0.06(0.01)
Kidney	710	148	2230	682	0.35 (0.05)
Fat*	120	77	160	131	0.56 (0.05)
Injection site L	17500	14300	17800	13300	0.86 (0.24)
Injection site R	13300	12000	12500	10000	0.96 (0.21)

Note: * fat samples were a composite of total fat isolated. R = right, L = left.

Chickens

Six broiler chickens, three males and three females, 5 to 5 weeks old and weighing 2.16-2.64 kg were dosed with ^{14}C-labelled flumequine for five consecutive days. The drug was administered orally as a solution by gavage into the crop at a dose of 18 mg/kg body weight. All animals were killed and exsanguinated 12 h after the final injection. The entire kidney, liver, breast and thigh muscle, omental fat pad and skin with fat were retained for HPLC analysis, determination of total radioactivity and microbiological activity.

The results of HPLC and radiochemical analysis are summarised in Table 5, showing that at the time of sacrifice about 30% of the radioactivity in liver is flumequine either as metabolites or bound residues. Metabolites or bound residues account for about 24% of the radioactivity in kidney, 6% in muscle, 23% in skin with fat. There was no measurable metabolism of flumequine in omental fat (Guyonnet, 1998c; Lynch and Speirs, 1998c).

Table 5. Ratio of flumequine to total radioactivity in chicken tissues after the oral administration of ^{14}C-labelled flumequine to broiler chickens daily for 5 consecutive days

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle	509	154	553	183	0.94 (0.10)
Liver	1080	397	1550	557	0.70 (0.04)
Kidney	1560	488	2060	624	0.76 (0.06)
Skin with Fat	275	95	361	127	0.77 (0.06)
Omental fat	129	38	123	44	1.09 (0.20)

Note: * fat samples were a composite of total fat isolated.

Trout

Two groups of 20 rainbow trout, with an average weight of 90.1 ± 8.1 g and 100.2 ± 10.3 g, respectively, were maintained in separate tanks with water temperatures of 7°C and 16°C, respectively. A single dose of ^{14}C-labelled flumequine was administered by gavage via a syringe, as a 2% formulation in lactose, enclosed in a gelatine capsule at a dose level of 12 mg/kg body weight. The method of administration was investigated prior to the study to confirm that the gelatine capsule ruptured in the stomach of the fish within 3 h of administration. Doses for individual fish were calculated based on weight at slaughter. Five trout from the group maintained at 16 °C were slaughtered at 18 h and five at 36 h after treatment and five trout from the group maintained at 7 °C were slaughtered at 36 h and five at 96 h.

The results of HPLC and radiochemical analysis are summarised in Table 6, showing that at the time of slaughter there is no evidence of metabolism of flumequine at any time point at either temperature (Guyonnet, 1998d, Caley, 1998).

Table 6. Ratio of flumequine to total radioactivity in trout muscle after the oral administration of a single dose of ^{14}C-labelled flumequine to trout maintained at different water temperatures

Tissue	Flumequine by HPLC		Total radioactivity, calculated as ^{14}C-flumequine		Ratio (± SD) of mean value of flumequine determined by HPLC and ^{14}C radioactivity
	Mean (μg/kg)	SD (μg/kg)	Mean (μg/kg)	SD (μg/kg)	
Muscle with skin, 16°C , 18 h, n = 8	3320	2210	3160	2030	1.04 (0.06)
Muscle with skin, 16°C , 36 h, n = 8	3160	1300	3110	1140	1.00 (0.07)
Muscle with skin, 7°C , 36 h, n = 8	3680	1750	3670	1490	0.98(0.11)
Muscle with skin, 7°C , 96 h, n = 8	1700	555	1760	585	0.97 (0.05)

METHODS OF ANALYSIS FOR RESIDUES IN TISSUE

A routine high performance liquid chromatographic (HPLC) analytical method for flumequine and its metabolite 7-hydroxyflumequine using fluorescence detection similar to the method presented to the 48th Committee was used to obtain data presented in the residue studies. Flumequine and 7-hydroxyflumequine were extracted from 2 gram sample with ethyl acetate without any prior deconjugation. Ibafloxacine was added to the sample as an internal standard to monotor retention time after clean up by liquid-liquid extraction. An extra step was performed for all samples with a high fat content where the extract was partioned between acetonitrile and hexane. Detection and quantification of flumequine was achieved by HPLC, employing gradient elution, using various mixtures of acetonitrile and 2.7 x 10^{-3} M aqueous oxalic acid (pH 2.5), on a C_{18} column using fluorescence detection with an excitation wavelength of 252 nm and an emision wavelength of 356 nm. Quantification was achieved by comparison of results with a calibration curve constructed from information obtained by the analysis of samples fortified with flumequine. The linearity, accuracy, repeatability, quantification and lower limits of detection of the method have been assessed in a single laboratory. No interlaboratory studies have been performed to test reproducibility. All data originate from one laboratory and one analyst. Within laboratory (day-to-day) precision data were all obtained from fortified blank tissues. No incurred tissues were included in the method validation. No ruggedness tests have been applied.

The specificity of the method was checked against some other quinolone antibiotics. Other quinolones tested for and showing no interference are oxolinic acid, nalidixic acid, marbofloxacin, danofloxacin, enrofloxacin and ciprofloxacin. Interference from "endogenous" compounds has been tested for each species/matrix combination with at least 20 "representative" blank samples.

The sponsor does not refer in the dossier as such to a routine regulatory analytical method for the determination of flumequine in edible tissues of pig, sheep, chicken, cattle and trout. However, in the dossier the HPLC-fluorescence-method(s) for the determination of flumequine are described that have been used to support residue depletion studies and are considered by the sponsor as potential regulatory methods (Guyonnet et al, 1996a and 1996b, CEVA, 2000). All these methods have the ability to determine the flumequine metabolite 7-hydroxyflumequine, however, no validation data for this application were supplied. The determination of 7-hydroxyflumequine was not carried out in studies discussed above.

All method descriptions are in conformity with ISO 78/2 Standard for drafting Standard Operating Protocols. The performance characteristics of the method, as they apply to pig and shep tissues, are detailed in Table 7 below (Guyonnet, 1998b, CEVA, 2000). The sponsor also provided similar data for calf and chicken tissues.

Table 7. The performance characteristics of routine high performance liquid chromatographic method for flumequine as determined for pig tissues

Validation parameter	Tissue							
	Kidney		Liver [b]		Muscle [a]		Skin/fat	fat[a]
	Pig[a]	Sheep[c]	Pig[b]	Sheep[c]	Pig[a]	Sheep[c]	Pig[a]	Sheep[c]
Range (µg/kg)	50-2500	5-100	50-2500	5-100	50-2500	5-100	50-2500	5-100
Precision (%)[e]	0.5-6.0	3.79-6.24	0.3-6.1	5.45-9.42	0.5-5.9	4.78-9.53	0.2-12.2	2.65-7.76
Recovery (%)	85.8-94.8	87.5[d]	76.1-93.7	78.3[d]	83.2-89.4	89.7[d]	78.9-91.4	83.8[d]
Accuracy (%)	-1.8/+5.5	-	-3.7/+0.2	-	-0.4/+6.6	-	-0.3/+8.7	-
LOQ (µg/kg)	50	5	50	5	50	5	50	5
LOD (µg/kg)	15	1	10	2	24	2	21	0.5

a = Samples homogenized with anhydrous Na_2SO_4, subsequent extraction with ethyl acetate.
b = Samples homogenized overnight with 1 molar HCl , subsequent extraction with ethyl acetate.
c= Samples homogenized in water with ultrasound, subsequent extraction with ethyl acetate.
d = "overall value"
e = day to day precision
- = no data supplied

APPRAISAL

The sponsor has provided the information on new residue studies that allow the estimation of the proportion of the total residues that accounted for parent drug at one time point for each species. The information provided was conducted to GLP and fully satisfies the provision of new data requested by the 48th Meeting.

The mean ratios of flumequine to total radioactivity in various tissues, derived from Tables 2-5, are summarised in Table 8. The new data provided show that:

- ratios of flumequine to total radioactivity in cattle kidney, muscle and fat (0.79 - 1.0) were somewhat above the average value of 0.5 estimated from residue depletion studies by the 48th Committee.

- The ratio of flumequine to total radioactivity in cattle liver (0.17) was somewhat below the average value of 0.25 estimated from residue depletion studies by the 48th Committee.

- ratios of flumequine to total radioactivity in sheep kidney, muscle and fat (0.35 - 0.56) were of the same order, or somewhat less than the value of 0.5 estimated from residue depletion studies by the 48th Committee used to recommend temporary MRLs.

- ratios of flumequine to total radioactivity in pig kidney, muscle and fat (0.44 - 0.75) were of the same order, or somwhat less than the value of 0.5 estimated from residue depletion studies by the 48th Committee and used to set temporary MRLs.

- ratios of flumequine to total radioactivity in pig and sheep fat (0..07 and 0.06, respectively) were about 4 times less than the value of 0.55 estimated from residue depletion studies by the 48th Committee and used to set temporary MRLs.

- there appears to be less metabolism of flumequine in chickens than in mammalian species. New data show ratios of flumequine to total radioactivity in chichen tissues (0.70 - 0.94), greater than the value of 0.5 estimated from residue depletion studies by the 48th Committee.

- there appears to be no measurable metabolism of flumequine in trout and correction factors (a ratio) does not need to be applied to residue data in considering appropriate MRL values

Table 8. Mean ratios of flumequine to total radioactivity in various tissues, derived from Tables 2 – 5.

Species	Tissue			
	Muscle	**Liver**	**Kidney**	**Fat**
Cattle	0.79	0.17	0.79	1.00
Pig	0.75	0.07	0.44	0.55*
Sheep	0.49	0.06	0.35	0.56
Chickens	0.94	0.70	0.76	0.77*
Trout	1.00**, 0.97***			

* = skin with fat ** = muscle with skin, 36 h, 16°C *** = muscle with skin, 96 h, 7°C

The sponsors method is suitable for regulatory purposes. Some methods, previously to other quinolone antibiotics (FAO, 1995), have also been applied to flumequine. Especially for *multi* quinolone residue analysis in food of animal origin a variety of useful HPLC methods has been published (Charriere et al, 1993; Ellerbroek, 1993; Haagsma et al, 1993; Heijden et al, 1993; Moretti et al, 1996; Samuelsen, 1990; Yorke, 1998) as well as planar chromatography (Juhel-Gaugain, 1996). For more efficient automated analyses aquous on-line dialysis and quinolone residue enrichment methods have been developed (Andresen & Rasmussen, 1990; Cohen et al, 1999; Eng et al, 1998; Leeuwen & Gend, 1989; Maxwell & Cohen, 1998). Also fast multi quinolone separation techniques are reported based on capillary zone electrophoresis (Perez-Ruiz et al, 1999; Sun & Chen, 1997). For final confirmation of test results in regulatory multi residue control an increasing number of methods based on hyphenated gas or liquid chromatography-mass spectrometry is available (Alvarez et al, 1997; Delepine et al, 1998; Doerge & Bajic, 1995; Plakas et al, 1999; Rose et al, 1998; Volmer et al, 1997; Munns et al, 1995; Pfenning et al, 1996).

If the Committee uses the same procedure to allocate MRLs to tissues of pigs, sheep, chickens and trout as it did to cattle, a problem arises with the data for the mean ratios of flumequine to total radioactivity in pig and sheep liver. Data provided suggests that since flumequine only accounted for about 6 - 7% of total residues, a correction factor (ratio) of 16 might be appropriate in TMDI calculations. Other data for pigs and sheep would suggest that the correction factor of 0.5 used for kidney, muscle and fat by the 48th Committee would be an appropriate figure to retain (with the possible exception of sheep kidney).

Reviewing the analytical data presented in Tables 3 and 4 for the determination of flumequine in pig and sheep liver, respectively, would suggest that it would not be possible to reliably quantify flumequine residues in sheep for more that two days after withdrawal of drug. This is because after 16 h, mean flumequine residues shown in Table 4 were only 2.5 times higher than the LOQ of the analytical method. Using similar logic, it should not be possible to reliably quantify flumequine residues in pigs for more than three days after withdrawal of drug.

MAXIMUM RESIDUE LIMITS

On the basis of the above considerations, the Committee recommended MRLs for edible tissues of cattle, pigs, sheep and chickens as follows: muscle and liver, 500 µg/kg; kidney, 3000 µg/kg and fat, 1000 µg/kg. The Committee also recommends 500 µg/kg for trout muscle, including skin in natural proportions. The MRL for flumequine in cattle liver and all temporary MRLs recommended by the Committee at its forty-eighth meeting are withdrawn.

Applying the values of food intake established by JECFA to the MRLs recommended above would result in a theoretical maximum daily intake of 1655 µg (see Table 9). This TMDI is based on residues in sheep, the species for which data indicates the most extensive metabolism of flumequine. This value is compatible with a maximum daily intake of 0-1800 µg

Table 9. Theoretical maximum daily intake (TMDI) of flumenquine residues

Food item	MRL (µg/kg)	Food Basket (g)	µg	MR/TR[1]	TMDI (µg)
Muscle	500	300	150	0.5	300
Liver	500	100	50	0.06	835
Kidney	3000	50	150	0.35	430
Fat	1000	50	50	0.55	90
Total					1655

Note: MR = marker residue; TR = total residue

REFERENCES

Alvarez E.J., Vartanian V.H., Brodbelt J.S. (1997). Metal complexation reactions of quinolone antibiotics in a quadrupole ion trap. Anal. Chem., 69;1147-1155.

Andresen A.T., Rasmussen K.E. (1990). Automated on-line dialysis and column switching HPLC determination of flumequine and oxolinic acid in fish liver. J. Liquid Chrom. Sci. 13; 4051-4065.

Caley, C.Y. (1998). Metabolism of [^{14}C] flumequine in rainbow trout. Inveresk Project Number 161882, Sanofi Dossier to the 54th JECFA Meeting.

CEVA SANTE ANIMALE S.A. (2000). Flumequine M.R.L. Worksheets for regulatory method. Sponsor submitted to the 54th JECFA Meeting.

Charriere R., Leiser W., Dousse R. (1993). HPLC methods for the determination of some quinolones in fish and animal tissues. In Haagsma N., Ruiter A. & Czedik-Eisenberg P.B. (Editors), Proceedings EuroResidue II Conference (1993) 241-245. ISBN 9061590167.

Cohen E., Maxwell R.J., Donoghue D.J. (1999). Automated multi-residue isolation of fluoroquinolone antimicrobials from fortified and incurred chicken liver using on-line microdialysis and high-performance liquid chromatography with programmable fluorescence detection. J. Chrom. B 724;137–145.

Delepine B., Hurtaud-Pessel D., Sanders P. (1998). Simultaneously determination of six quinolones in pig muscle by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Analyst 123; 2743-2747.

Doerge D.R., Bajic S. (1995). Multiresidue determination of quinolone antibiotics using liquid chromatography coupled to atmospheric-pressure chemical ionization mass spectrometry and tandem mass spectrometry. Rapid Commun. Mass. Spectrometry 9; 1012–1016.

Ellerbroek L. (1993). Microbial and chemical detection methods for residues of quinolones and related substances in animal tissue. In Haagsma N., Ruiter A. & Czedik-Eisenberg P.B. (Editors), Proceedings EuroResidue II Conference (1993) 280–284. ISBN 9061590167.

Eng G.Y., Maxwell R.J., Cohen E., Piotrowski E.G., Fiddler W. (1998). Determination of flumequine and oxolinic acid in fortified chicken tissue using on-line dialysis and high-performance liquid chromatography with fluorescence detection. J. Chrom. A 799; 349–354.

FAO (1998). Flumequine residue monograph prepared by the 48th meeting of the Committee and published in FAO Food and Nutrition Paper 41/10, Rome 1998

FAO (1996). Oxonilic acid residue monograph prepared by the 45th meeting of the Committee and published in FAO Food and Nutrition Paper 41/7, Rome 1996.

Guyonnet. J. (1998a). HPLC and microbiological determination of flumequine residues in tissues after subcutaneous administration of [^{14}C] flumequine to beef cattle for 5 consecutive days. Study Report MPK/Flumequine/9841, Sanofi Sante Nutition Animale, Sanofi Dossier to the 54th JECFA Meeting,

Guyonnet. J. (1998b). HPLC and microbiological determination of flumequine residues in tissues after intramuscular administration of [^{14}C] flumequine to pigs for 5 consecutive days. Study Report MPK/Flumequine/9839, Sanofi Sante Nutition Animale, Sanofi Dossier to the 54th JECFA Meeting,

Guyonnet. J. (1998c). HPLC and microbiological determination of flumequine residues in tissues after oral administration of [^{14}C] flumequine to broiler chickens for 5 consecutive days. Study Report MPK/Flumequine/9842, Sanofi Sante Nutition Animale, Sanofi Dossier to the 54th JECFA Meeting,

Guyonnet. J. (1998d). HPLC and microbiological determination of flumequine residues in edible tissue after single oral administration of [^{14}C] flumequine to rainbow trout. Study Report MPK/Flumequine/9843, Sanofi Sante Nutition Animale, Sanofi Dossier to the 54th JECFA Meeting,

Guyonnet. J. (1999). HPLC and microbiological determination of flumequine residues in tissues after intramuscular injection of [^{14}C] flumequine to sheep for 5 consecutive days. Study Report MPK/Flumequine/9838, Sanofi Sante Nutition Animale, Sanofi Dossier to the 54th JECFA Meeting,

Guyonnet J., Gouvello R. Le, Choubert D., Caizergues P., Bromet-Petit M., Spavone F., Thibaud D. (1996a). Flumequine residue depletion in rainbow trout maintained at two water temperatures after oral administration. In Haagsma N., Ruiter A. (Editors), Proceedings EuroResidue III Conference (1996) 446-450. ISBN 906159023x.

Guyonnet J., Pacaud M., Richard M., Doisi A., Spavone F., Thibaud D. (1996b). Routine determination of flumequine and its metabolite in edible tissues of pigs using an HPLC method. In Haagsma N., Ruiter A. (Editors), Proceedings EuroResidue III Conference (1996) 451-455. ISBN 906159023x.

Haagsma N., Roy M. van, Gortemaker B.G.M. (1993). High-performance liquid chromatographic determination of flumequine and its hydroxy metabolite in fish tissue. In Haagsma N., Ruiter A. & Czedik-Eisenberg P.B. (Editors), Proceedings EuroResidue II Conference (1993) 337-341. ISBN 9061590167.

Heijden van der M.H.T., Boon J.H., Nouws J.F.M., Mengelers M.J.B. (1993). Residue depletion of flumequine in European eel. In Haagsma N., Ruiter A. & Czedik-Eisenberg P.B. (Editors), Proceedings EuroResidue II Conference (1993) 357-361. ISBN 9061590167.

Juhel-Gaugain M.. (1996). Screening of quinolone residues in pork muscle by planar chromatography. In Haagsma N., Ruiter A. (Editors), Proceedings EuroResidue III Conference (1996) 578-581. ISBN 906159023x.

Leeuwen van W., Gend H.W. van (1989). Automated determination of flumequine in whole egg, milk and meat via continuous flow / high performance liquid chromatography (CF/HPLC) [in Dutch] *Geautomatiseerde bepaling van flumequine in heelei, melk en vlees met behulp van continuous flow/hoge prestatie vloeistofchromatografie (CF/HPLC).* RKvW Utrecht, NL. Report IR/73/03/89/d33, pp 17.

Lynch, C.J. and McLean, C.L. (1998). Tissue residues of total radioactivity following subcutaneous administration of [^{14}C] flumequine to beef cattle for five consecutive days. Inveresk Project Number 161861, Sanofi Dossier to the 54th JECFA Meeting,

Lynch, C.J. and Speirs, G.C. (1998a). Tissue residues of total radioactivity following intramuscular administration of [^{14}C] flumequine to pig for five consecutive days. Inveresk Project Number 161882, Sanofi Dossier to the 54th JECFA Meeting,

Lynch, C.J. and Speirs, G.C. (1998b). Tissue residues of total radioactivity following intramuscular administration of [^{14}C] flumequine to sheep for five consecutive days. Inveresk Project Number 161877, Sanofi Dossier to the 54th JECFA Meeting,

Lynch, C.J. and Speirs, G.C. (1998c). Tissue residues of total radioactivity in tissues following oral administration of [^{14}C] flumequine to broiler chickens for five consecutive days. Inveresk Project Number 162074, Sanofi Dossier to the 54th JECFA Meeting,

Maxwell R.J., Cohen E. (1998). Comparison of programmable *versus* single wavelength fluorescence for the detection of three fluoroquinolone antibacterials isolated from fortified chicken liver using coupled on line microdialysis. J. High Resol.Chromatogr. 21; 241-244.

Moretti V.M., Luzzana U., Albertini A., Valfre F. (1996). Determination of 4-quinolone antibiotics by high performance liquid chromatography. In Haagsma N., Ruiter A. (Editors), Proceedings EuroResidue III Conference (1996) 720-725. ISBN 906159023x.

Munns R.K., Turnipseed S.B., Pfenning A.P., Roybal J.E., Holland D.C., Long A.R., Plakas S.M. (1995). Determination of residues of flumequine and nalidixic, oxolinic, and piromidic acids in catfish by liquid chromatography with fluorescence and UV detection. J. AOAC Intl.78; 343-352.

Perez-Ruiz T., Martinez-Lozano C., Sanz A., Tomas V., Bravo E. (1999). Separation and simultaneous determination of quinolone antibiotics by capillary zone electrophoresis. Poster abstract 23rd Symposium HPLC-99, 30 May-4 June, Granada, ES (1999) pp 1.

Pfenning A.P., Munns R.K., Turnipseed S.B., Roybal J.E., Holland D.C., Long A.R., Plakas S.M. (1996). Determination and confirmation of identities of flumequine and nalidixic, oxolinic, and piromidic acids in salmon and shrimp. J. AOAC Intl., 79; 1227-1235.

Plakas S.M., El-Said K.R., Bencsath F.A., Musser S.M., Walker C.C. (1999). Determination of flumequine in channel catfish by liquid chromatography with fluorescence detection. J. AOAC Intl.,82; 614-619.

Rose M.D., Bygrave J., Stubbings G.W.F. (1998). Extension of multi-residue methodology to include the determination of quinolones in food. Analyst 123; 2789-2796.

Samuelsen O.B. (1990). Simple and rapid method for the determination of flumequine and oxolinic acid in salmon (*Salmo salar*) plasma by high performance liquid chromatography and fluorescence detection. J Chromatograph.. 530; 452-457.

Steffenak I., Hormazabal V., Yndestad M. (1991). Reservoir of quinolone residues in fish. Food Add. Contam. 8; 777-780

Steffenak I., Hormazabal V., Yndestad M. (1993). Residues of quinolones in fish and the effect of cooking on the residues in the fish. In Haagsma N., Ruiter A. & Czedik-Eisenberg P.B. (Editors), Proceedings EuroResidue II Conference (1993) 646 – 649. ISBN 9061590167.

Sun S.-W., Chen L.-Y. (1997). Optimization of capillary electrophoretic separation of quinolone antibacterials using overlapping resolution mapping scheme. J. Chrom. A 766; 215–224.

Volmer D.A., Mansoori B., Locke S.J. (1997). Study of 4-quinolone antibiotics in biological samples by short-column liquid chromatography coupled with electrospray ionization tandem mass spectrometry. Anal. Chem. 69; 4143–4155.

Yorke J.C. (1998). High performance liquid chromatographic method with fluorescence detection for the quantification of nine quinolones in chicken tissues. Poster abstract D41, 3rd International symposium on hormone and veterinary drug residue analysis, 2-5 June, Brugge, BE (1998).

IVERMECTIN

First draft prepared by
Dieter Arnold, Berlin, Germany
Robert Wells, New South Wales, Australia

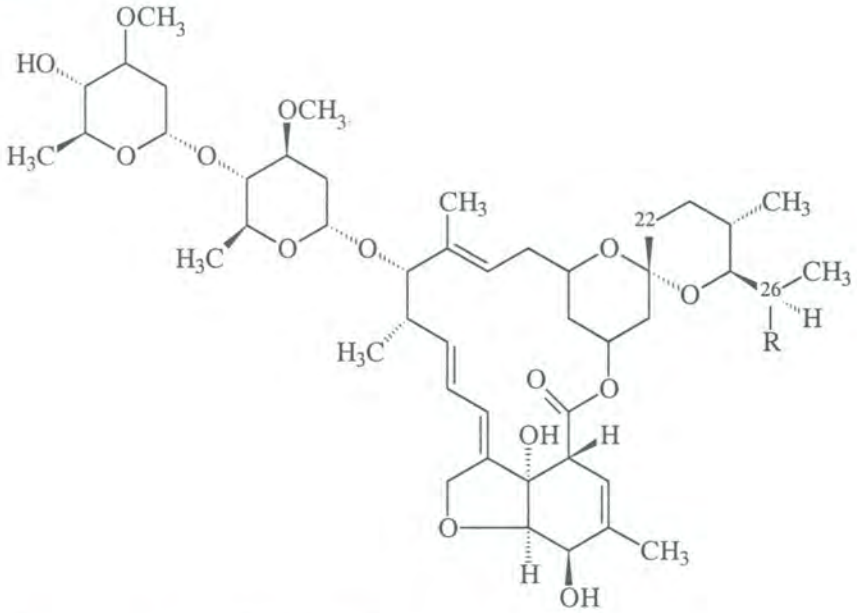
ADDENDUM
to the Ivermectin residue monographs
prepared by the 36th and 40th meetings of the Committee and published in
FAO Food and Nutrition Papers 41/3, Rome 1991 and 41/5, Rome 1993

IDENTITY

Chemical name: 5-O-Demethyl-22,23-dihydroavermectin-A_{1a}
5-O-Demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl) avermectin-A_{1a}; 22,23 dihydroavermectin B_{1b} (Chemical Abstracts name)
CAS Number: 7-288-86-7

International Nonproprietary Name: Ivermectin

Structural formula:



Molecular formula: Component **B_{1a}:** $C_{48}H_{74}O_{14}$
Component **B_{1b}:** $C_{47}H_{72}O_{14}$

Molecular mass: Component **B_{1a}:** 874
Component **B_{1b}:** 860

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Off-white powder
Crystals from ethanol/water, m.p. 154.5 - 157°C

Optical rotation: $[\alpha]_D$: $+71.5 \pm 3°$ (c=0.755 in chloroform)

Ultraviolet-maxima: 238 nm (ε = 27100), 245 nm (ε = 30100) in methanol

Solubility: Solubility in water: 0.006-0.009 mg/L

INTRODUCTION

Ivermectin is widely used as a broad-spectrum antiparasitic drug against nematodes and arthropods in food-producing animals. In human medicine, it is mainly used for the treatment of *onchocerciasis*. Ivermectin was previously reviewed by the Committee at its thirty-sixth and fortieth meeting (Annex 1, reference 91, Annex 1, reference 104). At the 40th meeting the Committee recommended an ADI of 0-1 µg/kg of body weight per day and MRLs for ivermectin B_{1a} in cattle of 100 µg/kg in liver and 40 µg/kg in fat. At its present meeting, the Committee reviewed additional studies in which the drug was used topically in dairy cows. The recommended dose is 0.5 mg/kg of body weight. The drug is used as a solution (5g/L).

RESIDUES IN MILK AND THEIR EVALUATION

Six lactating Friesian dairy cows, were treated with a single dose of approximately 580 µg/kg of body weight (**O'Neill**, 1997). The characteristics of the animals, including treatment and performance are summarized in Table 1. The cows were grazing on irrigated pasture. During milking they were fed a specially formulated supplement. Milk samples were collected twice daily and were analyzed using a method with a postulated limit of detection (LOD) of 1µg/kg of milk for both Ivermectin B_{1a} and Ivermectin B_{1b}. Method recoveries were as given in Table 2. The results were corrected for recoveries.

Table 1. Characteristics of the Friesian cows and their performance and depletion of residues in milk

Friesian Cow Study						
Animal identification number	1	2	3	4	5	6
Number of lactations	2	3	6	5	5	8
Days in milk	134	125	128	127	99	125
Body weight [kg]	605	555	600	640	555	615
Dose [µg/kg of body weight]	579	586	583	578	586	585
Dose [mg/animal]	350	325	350	370	325	360
Milk yield [kg][a)]	247	311	289	264	279	298
Milkfat secreted [kg][a)]	10.2	11.7	10.4	11.0	10.6	9.9
Residues excreted [µg][a)]	784	579	756	927	832	990
% of dose excreted[a)] via milk	0.22	0.18	0.22	0.25	0.26	0.28

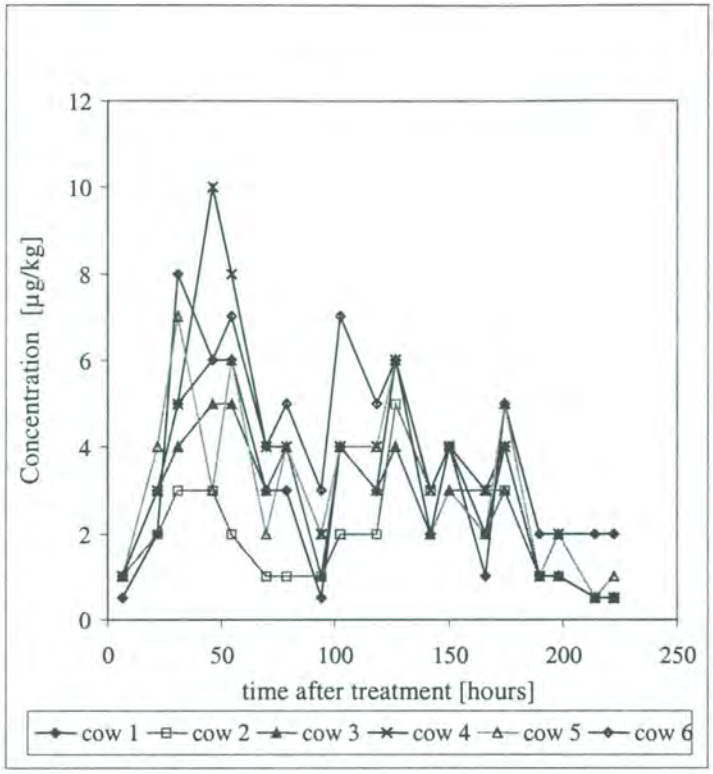
Note: [a)] summarized over the first 222.5 hours after treatment

Table 2. Recoveries of the analytical method used for the determination of Ivermectin B_{1a} in milk

Method recoveries for Ivermectin B_{1a} in milk				
Fortified level [µg/kg]	N	mean recovery [%]	standard deviation [%]	CV [%]
5	6	83	8.3	10
25	6	88	4.4	5
50	6	96	5.8	6

The concentrations of ivermectin B_{1a} in milk reached a maximum in samples obtained at the third or forth milking after treatment; subsequently one or more, usually broader, maxima, were reached. The later maxima were typically lower than the first maximum, except in milk obtained from one cow in which the highest concentration was reached at the tenth milking, about 130 h after treatment of the cow. The results are given in Figure 1

Figure 1. Milk excretion data on ivermectin H_2B_{1a} residues in Friesian cows treated with a single topical dose.



A similarly designed study was carried out using 3 - 6 year old lactating Jersey cows, 17 - 52 days in milk and treated with Coopers Paramax Pour-on for Cattle (**O'Neill** et al., 1998). This study was also carried out in a different geographical region. The animals were grazing on pasture. They were offered silage *ad libitum* in the paddock and received 5 kg of barley per day, split equally at each milking. The characteristics of the animals, including treatment and performance are summarized in Table 3. Milk samples were collected twice daily. Analyses were carried out using the same methodology as indicated for the study using Friesian cows.

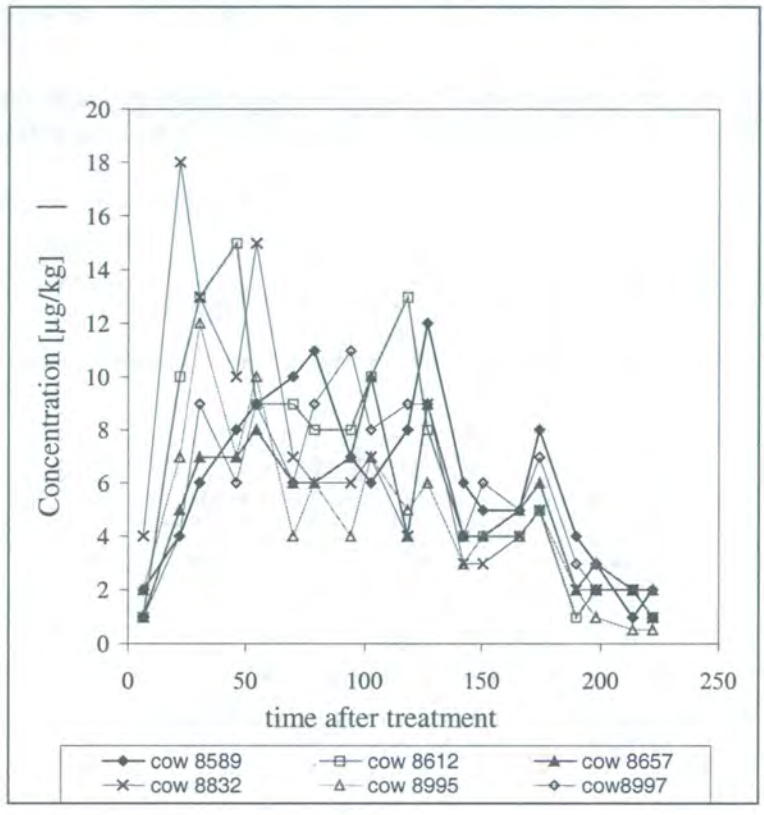
Table 3. Characteristics of the Jersey cows and their performance and depletion of residues in milk.

Jersey Cow Study						
Animal identification number	8612	8995	8832	8997	8657	8589
Number of lactations	4	3	4	3	4	4
Days in milk	26	36	45	36	17	52
Body weight [kg]	406	369	432	390	425	401
Dose [µg/kg of body weight]	579	583	579	577	576	586
Dose [mg/animal]	235	215	250	225	245	235
Milk yield [kg][a)]	202	149	191	161	187	142
Milkfat secreted [kg][a)]	12.9	7.39	11.8	9.21	10	9.13
Residues excreted [µg][a)]	1368	665	1203	923	908	868
% of dose excreted[a)] via milk	0.85	0.31	0.48	0.41	0.37	0.37

Note: [a)] during the first 222.5 hours post-dose

In this second study milk yield was lower than in the first one, fat excretion was only slightly lower and residue excretion was significantly higher. Thus, a significantly higher proportion of the administered dose was excreted via milk. The kinetics of depletion of residues was similar (see Figure 2), but the maxima in the milk of most animals, were higher. The highest concentrations of ivermectin B_{1a} in the milk of individual animals during the period of observation was 5-10 µg/ kg of milk in the study of Friesian cows. In the study of Jersey cows the range of the maxima was 10-18 µg/kg of milk. The contribution to the total residue of the concentrations of ivermectin B_{1b} was insignificant in both studies and typically below the reported limit of detection.

Figure 2. Milk excretion data on ivermectin H_2B_{1a} residues in Jersey cows treated with a single topical dose.



METHOD OF ANALYSIS

A method for the identification and quantification of ivermectin B_{1a} and ivermectin B_{1b} residues in milk was submitted (**Agal**, 1998). Ivermectin residues are extracted from milk by homogenisation with acetonitrile and evaporation of the acetonitrile under vacuum. The residue is dissolved in a mixture of hexane and dichloromethane; this solution is applied to a silica gel cartridge and ivermectin residues are eluted from the cartridge with ethyl acetate. The ethyl acetate is removed with a stream of nitrogen. The dry residue is treated with a mixture of 1-methylimidazole and acetic anhydride in dimethylformamide, the derivatisation mixture is cleaned up by application to a C_{18} cartridge and washing of the cartridge with 50% aqueous methanol. The fluorescent ivermectin derivatives are then eluted with methanol. Methanol is removed under vacuum and the residue re-dissolved in a known volume of methanol.

HPLC determination is performed on a C_{18} HPLC column, using an elution solvent of 95% aqueous methanol and fluorescence detection (360 nm excitation, 468 nm emission). Under these conditions, total analysis time is about 15 minutes and the derivatives formed from ivermectin B_{1a} and B_{1b}, respectively, are well separated

This method was not developed under GLP, and an incomplete set of the required validation data was made available by the sponsor. Neither the limits of detection (LOD) nor the limits of quantification (LOQ) for the two compounds was determined. The recovery of the method was estimated at concentrations of 5, 25, and 50 µg/kg of ivermectin B_{1a} and 2 and 4 µg/kg of ivermectin B_{1b} (see the above Table 2); however, many of the milk samples obtained in the studies of depletion had lower concentrations.

MAXIMUM RESIDUE LIMITS AND INTAKE ASSESSMENT

The ADI established at the 40th meeting of the Committee was 0-1 µg/kg body weight, equivalent to a maximum of 60 µg per person. Taking into account the TMDI of 39.4 µg/person, as estimated for daily consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, and 50g of fat and the MRLs recommended at the 40th Meeting, a TMDI of 21 µg/person represents the highest allowable intake from the consumption of 1.5 kg of milk which would not result in a total TMDI in excess of the ADI. A concentration of ivermectin B_{1a} of 10µg /kg of whole milk would result in a TMDI of 10 x 1.5 =15µg/person for a daily consumption of 1500 g of milk.

On this basis the Committee recommended a temporary MRL of 10 µg for ivermectin B_{1a}/kg of whole milk in cattle. It was aware that with the currently used formulation of the drug this MRL would require a milk discard time of up to 11 milkings. However, the recommended MRL could serve also as a basis to develop other formulations and/or other conditions of use.

REFERENCES

AGAL (1998). Determination of macrocyclic lactones (ivermectins) in milk using pre-column derivatisation and fluorescence detection. Australian Government Analytical Laboratories Method, Version Number 6, Date of issue, 1998

O'Neill, P. (1997) A research report dealing ivermectin residue levels in whole milk following treatment of lactating dairy cattle with a 5.0 g/L ivermectin pour-on formulation for cattle. Doc. No. 97/0633; Schering-Plough Animal Health Limited.

O'Neill, P., Baum, K., and Ho, K.B. (1998) A research report dealing ivermectin residue levels in whole milk following treatment of lactating Channel Island breed dairy cattle with 5.0 g/L ivermectin pour-on formulation for cattle. Doc.No. 98/0540; Schering-Plough Animal Health Limited.

LINCOMYCIN

First draft prepared by

Barbara Röstel, Fougères, France
Jan Zmudzki, Pulawy, Poland
James MacNeil, Saskatchewan, Canada

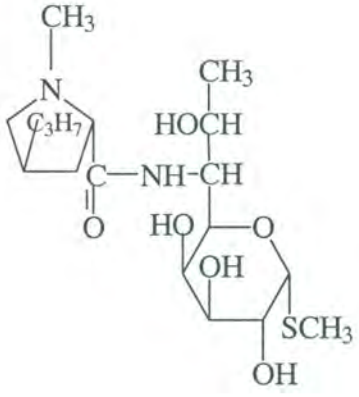
IDENTITY

Chemical name: Methyl 6,8-dideoxy-6-[[(1-methyl-4-propyl-2- pyrrolidinyl)carbonyl]amino]-1-thio- D-erythro-α-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-propyl-L-2-pyrolidone-carboxamido)-1-thio-D-erythro-α-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: 406.6

OTHER INFORMATION ON IDENTIY AND PROPERTIES

Appearance: White to tan to brown crystalline powder, color depending on grade of material. The high grade used in injectable products is whiter than the grades used in premixes.

Melting point: 145 – 147 ° C

Solubility: Lincomycin hydrochloride: Soluble in water, (1:1) in ethanol, (1:40) in dimethylformamide, (1:20), in methanol. Very slightly in acetone and practically insoluble in chloroform and ether

Optical rotation: Licomycin hydrochloride (1% in water) $[\alpha]^{25}_{D} = +142°$

Ultraviolet maxima: none

Stability: Lincomycin is a very stable molecule and does not degrade under normal environmental conditions.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Lincomycin is an antibiotic produced by *Strepromyces linconensis.* It belongs to the class of lincosamides, which are derivatives of an amino acid and a sulfur containing galactoside. Other substances belonging to the lincosamide group are clindamycin and pirlimycin.

Lincomycin is used as oral preparation in feed and water in poultry for treatment of bacterial enteric infections and as a performance enhancer; in pig it is used for treatment and control of bacterial enteric, mycoplasmal respiratory infections, in infectious arthritis and as a performance enhancer. Mono preparations are also used as parental formulations in pig for treatment of bacterial enteric, mycoplasmal respiratory infections and in infectious arthritis.

Combination-preparations with spectinomycin are used in poultry for oral application (water) for treatment and control of respiratory disease and increase in weight gain. In pig oral preparations (water, feed) are intended for treatment and control of enteric and respiratory disease, treatment of infectious arthritis and increase in weight gain. Parental preparations (i.m.) exist for treatment of bacterial enteric and respiratory disease in pig and calves and for treatment of arthritis in pig and contagious foot-rot in sheep. The combination-preparation with sulfadiazine is used orally (feed) in pig for treatment of atrophic rhinitis and enzootic pneumonia. Combination preparations with neomycin are used as intramammary applications in lactating dairy cattle for treatment of acute mastitis.

Dosage:

Table 1. Maximum daily Lincomycin doses per animal species and route of administration (mg/kg body weight or mg/mammary quarter).

Species	Oral	Parental	Intramammary
Poultry	50 mg/kg bw		
Pig	13 mg/kg bw	10 mg/kg bw	
Calves		5 mg/kg bw	
Sheep		5 mg/kg bw	
Dairy Cows			600 mg/quarter

PHARMACOKINETICS AND METABOLISM

Laboratory Animals

Rat

Adult male rats received one single i.m. lincomycin dose of 0 and 100 mg/kg body weight (Davis, Balcolm 1969). Groups of 10 rats were killed at 15, 30 minutes, 1, 1.5, 3, 6 and 8 hours after treatment at which time blood and bone samples were collected. Lincomycin is rapidly absorbed and plasma levels reach mean peak concentrations of 20.9 mg/kg and declined biphasically to concentrations of 2.5 mg/kg at 8 hours.

Dog

One dog (female, 11.5 kg) received one single dose of tritium labelled lincomycin hydrochloride of 500 mg equivalents once i.m. and once orally (Eberts et al. 1963). After oral application peak plasma concentrations of 4.5 mg/kg were reached at 4 hours. The plasma half-life was 4.1 hours. Of the administered dose, 14% was excreted in urine and 77% in feces. Total recovery of the dose was 92%. The amount of oral absorption was calculated as 35%. The half life curves of urinary excretion was biphasic with values of 4.3 and 27.4 hours. After i.m. application, peak plasma concentrations of 25.2 mg/kg were reached within 10 minutes, with a half-life of 4 hours. Of the 87% of radioactivity recovered, 49% were excreted in urine and 38% in feces. The biphasic half-life curve of urinary excretion had values of 3.8 and 20.4 hours. Urinary excretion for both routes of application was essentially complete in less than 24 hours and in fecal excretion, within 48 hours.

Food Animals

Pig

Serum time curves were determined in 3 pigs after a single i.m. dose of 11 mg lincomycin hydrochloride/kg bw (Russel 1979). Blood samples were taken at 0.5, 1, 1.5, 2, 2.5, 4, 6 and 8 hours and analysed by the microbiological assay.

Plasma concentrations peaked between 0.5 and 1 hour after treatment with maximum level ranging between 5.33 and 10.92 mg/kg and gradually declining over 8 hours.

In a GLP conforming study, five groups of two pigs received oral doses of ^{14}C lincomycin hydrochloride of 440 mg per animal per day by capsule (Hornish, Gosline 1981). Capsules were given every 6 hours. One group received 6 doses, one 14 doses and three groups received 10 doses. The groups treated with 6 and 14 doses were killed 4 hours post treatment, and the groups treated with 10 doses were killed 4, 48 and 96 hours post treatment. Blood and tissue samples were analysed. Total ^{14}C accountability was 96%. Highest concentrations were found in liver and kidney samples of the 10 dose treatment animals with mean levels of 14.0 mg/kg-equivalents in liver and 10.1 mg/kg-equivalents in kidney followed by muscle (0.7 mg/kg-equivalents) and fat (<0.1 mg/kg-equivlents) depleting to 1.01 mg/kg-equivalents in liver and 1.1 mg/kg-equivalents in kidney at 96 hours.

In a GLP study, one group of 7 pigs received a single i.v. dose of 10 mg linocmycin hydrochloride/kg bw. After a one week treatment-free interval, the same pigs received the same dose once orally by capsule (Hornisch, et al., 1985). Blood samples were taken at 0, 0.1, 0.2, 0.3, 0.5, 1, 2, 4, 8, 12 and 16 hours after i.v. treatment and at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 8, 12 and 16 hours after oral treatment and the residues examined by microbiological methods. The oral absorption in pig was 53 ± 19%. Plasma protein binding at blood concentrations of 0.5 to 20 mg/kg was 5-15%. The absorption and bioavailability follows a first order kinetic profile. The distribution and excretion for the oral application represents a one compartment and first order elimination. For the i.v. application, a biphasic, two compartment model with an initial fast elimination through the first 0.5 hours followed by a slower terminal elimination phase was observed. C_{max} was determined as 1.45 mg/kg at the t_{max} of 3.6 hours for the oral dose. The large variation coefficient of 48% for C_{max} was explained by the profound effect of food intake exerted on the absorption. The mean $t_{1/2}$ was calculated as 3.36 ± 1.27 for the oral route and 1.95 ± 0.42 for the parental route.

Groups of 4 pigs received a single i.m. dose of lincomycin hydrochloride of 2, 5 and 10 mg/kgb bw, corresponding to 4.4, 11, 22, mg/kg bw per day (Barbiers, Kleckner 1964). Blood samples were taken at 0, 1, 2, 4, 8, 12, 24, 36 and 48 hours after treatment. Dose dependent maximum concentration were seen at 1 hour declining at 16 hours to the limit of quantification (LOQ = 0.1 mg/kg) of the microbiological method. Additionally, groups of 3 pigs received a single oral dose of lincomycin hydrochloride of 10, 25 and 50 mg/kgb bw corresponding to 22, 55, 110 mg/kg bw. The same sampling schedule was applied. Dose related maximum blood concentrations were found at 4 hours after treatment declining at 24 to 36 hours to the LOD of the method.

Two groups of 3 pigs received daily i.m. doses of lincomycin hydroxhloride of 11mg/kg bw, in one group for 3 days, the other group received treatment for 7 days (Barbiers et al 1975). One hour after the last treatment, injection site, muscle, fat, kidney, liver, blood, urine and other tissues and body fluid were collected and analysed by the microbiological method. Lincomycin is absorbed from the injection site and distributed to the various tissues and body fluids. Highest values were found in urine (413 mg/kg in the 3 day treatment group and 261 mg/kg in the 7 day treatment group) confirm very rapid excretion. Plasma concentrations were 6.07 mg/kg (3day treatment group) and 3.80 mg/kg (7day treatment group). In tissues the highest concentrations were seen in injection sites (14.66 mg/kg (3 days) and 93.33 mg/kg (7 days), followed by kidney (21 mg/kg in 3day treatment group and 15 mg/kg in the 7day treatment group), liver (8.43 mg/kg (3days) and 6.40 mg/kg (7days),in muscle, 6.07 mg/kg (3days) and 3.80 mg/kg (7days), and in fat (2.19 mg/kg (3days) and 2.17 mg/kg (7days).

One group of 3 pigs received daily oral doses of 22 mg lincomycin hydrochloride/lb bw for 3 days, a second group of 3 pigs received daily i.m. doses of 22 mg lincomycin hydrochloride/lb bw for 3 days (Barbiers, Kleckner 1964a, 1964b). Blood samples were taken at 0, 1, 2, 4, 8, 12, 24, 36, 48, 60 and 72 hours after treatment. Tissue samples from one pig of each group were taken at 1, 2 and 3 days after treatment. Blood concentrations were comparable to the previous trials with no detectable residues after 24 hours. In tissues, the only residues were found in liver and kidney in the 24 hour samples with higher concentrations found tissues of orally treated animals (liver, 1.1 mg/kg, in kidney 1.6 mg/kg) as measured by a microbiological method.

Four groups of six pigs received lincomycin in feed in concentrations of 0 and 44 mg/kg (DeGeeter, Barbiers 1978). Animals were killed at 0, 1 and 2 days after withdrawal of medication. Blood and tissue samples were analysed by bio-assay. Lincomycin was detected only in liver and kidney samples with values for liver ranging from <0.1 to 0.12 mg/kg and higher values for kidney, from 0.14 to 0.28 mg/kg. No lincomycin was detected in any of the samples at 1 and 2 days withdrawal. The LOQ of the method is 0.1 mg/kg.

Chicken

One group of 8 chickens received twice daily oral doses of 0.47-0.76 mg/kg bw ^{14}C lincomycin hydrochloride/kg bw per capsule for 12 days after having been exposed for 36 day to non labelled lincomycin hydrochloride in feed at a concentration of 10 g/ton (Gosline et al., 1978). Blood, offal, bile and tissues from two chickens were collected for

analysis at 1 hour, 1, 2, and 3 days after final treatment. On-treatment excreta contained 90.2 ± 8.6 % of the administered radioactivity. The half-life in bile and offal were 8.3 and 11.3 hours, respectively. Only liver samples at 1 hour after treatment contained detectable residues (LOD = 0.1 mg/kg), however, the residues were biologically inactive.

Dairy cattle
One first-calf heifer in mid lactation received a single i.v. dose of 11 mg lincomycin/kg bw (Weber et al,. 1981). Blood samples were taken frequently up to 48 hours. Milk samples were taken at 0, 4, 8, 12, 18, 24, 36 and 48 hours. Urine samples were taken in 20 minute intervals up to 4 hours and at 6, 7, 8, 12, 18, 24, 36, 48, 60, and 72 hours. The same animal was used in two trials using an intramammary infusion of 5 mg lincomycin/lb, equally applied to all quarters. In the first trial, blood samples were taken very frequently up to 48 hours, milk samples at 0, 24, 36, 48, 60, and 72 hours. Urine samples were taken in 20 minute intervals up to 3 hours and at 8, 12, 18, 24, 36, 48 and 72 hours. In the second trial, milk samples were taken at one hour intervals through 12 hours and at 16, 20, 36, 48, 50 and 72 hours. Additionally, using the same experimental design, two first-calf heifers in mid lactation received a single i.v. dose of lincomycin of 5.5 and 11 mg/kg bw, respectively. Blood, milk and urine sampling were the same as in the first study.

Lincomycin in cows shows biexponential pharmacokinetics and represent first order kinetics. Intravenous doses of 5.5 and 11 mg/kg bw give linear kinetics. An approximately constant 32% of the dose is excreted in the urine, independent of the route of administration. Only 1.5% of the i.v. dose is excreted in the milk; 85% of an intramammary dose is absorbed into the blood. Changes in the rate of milk production are shown to strongly influence the kinetics of transfer of lincomycin into and out of the udder. Approximately 65% of the dose, regardless of the route of administration is metabolised to bio-inactive metabolites.

Man
Five healthy men received lincomycin as an oral dose of 0.5 and 1.0 g/person (64-104 kg) and as an i.m. dose of 0.6 g/person (Kaplan et al., 1965). The drug was given in a fasting state and after a meal. Blood samples were taken before and 1, 2, 4, 6, 8, 12, and 24 hours after treatment. Urine was collected for 24 hours. After the 0.5 g dose, plasma concentrations were higher in the fasting males and peaked with 1.4 to 1.8 mg/kg at about 4 hours and were maintained at the higher concentrations when compared to non-fasted individuals over a period of 12 hours. Lincomycin levels after eating are lower in plasma with values of 0.6 - 0.7 mg/kg over 2 to 8 hours. About 4 to 7% of the administered dose is excreted in the urine within 24 hours. After i.m. injection, peak plasma concentrations appeared at 1 - 2 hours with values of 6.8 - 11.6 mg/kg decreasing gradually to 1 mg/kg at 24 hours. Approximately 30 to 60% was excreted in urine within 24 hours.

METABOLISM

The metabolism of lincomycin in dog, chicken and pigs has been described. Comparative studies of the metabolism of lincomycin in pigs and rats and in chicken and rats were provided.

Laboratory Animals

Dog
Three beagle dogs received a single i.v. dose of ^{14}C-lincomycin hydrochloride equivalent to 100 mg lincomycin free base per animal 7.7-8.5 mg/kg bw (Daniels and Van Eyk, 1978). Urine and feces were collected continuously for 101 hours. Recovery of radioactivity was complete with 95% of the administered dose accounted for. Biliary excretion plays the predominant role with 55 - 60% of radioactivity excreted in feces and 32 - 40% in urine. Thin layer chromatography (TLC) was used for identification of parent lincomycin, which accounted for 17% of the administered dose excreted in feces and 28.5% in urine. Very small amounts (<3% of the dose) of lincomycin sulfoxide and N-demethyllincomycin were found.

Food Animals

Pig
In pigs, metabolism was rapid and extensive, leading to 26 metabolites in liver. Except for the parent compound, none of the metabolites has been characterized, and each was present at a concentration representing less than 10% of the radiolabelled material (Hornish et al., 1987). In a comparative analysis of microbiological and gas chromatographic-mass spectrometric (GC/MS) methods, lincomycin appeared to account for all of the microbiologically active residues in pig liver and kidney (Nappier et al., 1989, Nappier et al., 1996f).

Comparative metabolism studies were conducted in pigs and rats (Hornisch et al. 1987). Pigs were treated orally (capsules) at doses of 15 mg/kg bw/day, rats at doses of 300 mg/kg bw/day of ^{14}C-lincomycin in drinking water. Of the

total dose administered, 5% was excreted in urine of rats, whereas 14-21% was found in pig urine. Feces and GI tract content contained the major portion of excreted drug in rats (95%) and in pigs (79-86%). All major components separated by HPLC in pig urine were also qualitatively found in rat urine. Analysis of feces demonstrated significant differences between the two species in a quantitative sense but only minor differences on a qualitative basis. Most fecal metabolites found in pig were also found in rats. In liver samples the ratio of the final polar fraction to the chloroform fraction was 2:1 in rats and 5:1 or higher in pigs indicating a relatively higher content of lincomycin in rat liver. Although quantitative differences were found in the different analysed fractions there was greater than 90% qualitative match of pig liver metabolites to rat liver metabolites.

Chicken

Comparative metabolism studies were conducted in chicken and rats (Hornisch et al., 1987). Chickens received doses of 5.5-6.5 mg/kg bw/day of ^{14}C-lincomycin for 7 days. Lincomycin was the major excreta metabolite in chickens with 60-80% on treatment and about 50% 2-4 days after treatment. Small amounts of N-demethyllincomycin and lincomycin sulfoxide were identified only during treatment. An unknown metabolite comprising 10% during treatment and increasing to 50% at 4 days after treatment was considered to be generated by gut microflora of the chicken. In these comparative studies of the metabolism of lincomycin in chicken and rat liver, it was found to be qualitatively similar, although the metabolites were not identified.

Twenty-one female and 21 male broiler chickens (35 days old) were exposed to doses of 128 mg/gal (5.1 to 6.6 mg/kg bw/day) ^{14}C-lincomycin hydrochloride in drinking water for 7 days; six animals (3 male, 3 female) served as control (Hornisch et al. 1984). Total radioactive residue concentrations were measured in liver, kidney, muscle and skin and fat at 0, 0.5, 1, 2, 4, and 7 days after treatment. Liver and kidney contained the highest total residue concentrations, declining in liver from 1.58 (day 0) to 0.02 mg/kg-equivalents (day 7), and in kidney from 1.26 (day 0) to 0.01 (day 7) mg/kg-equivalents. Total residue concentrations declined in muscle from 0.05 (day 0) to <0.05 (day 2) mg/kg-equivalents and in skin/fat from 0.13 (day 0) to <0.05 (day 7) mg/kg-equivalents. At 0 hours 75% of total liver residues were identified as: lincomycin (20%), lincomycin sulfoxide (40%), N-demethyllinocmycin (5%), and N-demethyllincomycin sulfoxide (10%). In muscle, lincomycin (16%) and one unknown, metabolite VI, (37%) accounted for more than 50% of the total residues at 0 hours. In skin and fat, corresponding values were: lincomycin (18%) and unknown metabolite VI (11%); they comprised about 40% of total residues at 0 hours. During treatment excreta contained 60-85% unmetabolised lincomycin declining slightly to 50-55% of total residues at 4 days after treatment. Lincomycin sulfoxide (6-10%), N-methyllincomycin (3-6%) and one unknown (10%) were the remaining on-treatment excreta residues.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

The depletion of radiolabelled ^{14}C lincomycin was studied in pigs and chicken.

Pig

In a study conducted prior to GLP, groups of six pigs were fed diets containing ^{14}C-lincomycin at concentrations equivalent to 1.2, 2.0, 6.0-7.0, or 10-12 mg/kg of body weight per day for 3 days and were killed 12 h after the last treatment (Hornish et al., 1987). An additional group of six pigs received the maximum dose for 3 days and were killed 48 h after treatment. The mean concentrations of total residues at 12 h for the four different treatments were 0.40, 0.64, 1.6, and 3.4 mg/kg in liver; 0.22, 0.41, 1.2, and 3.1 mg/kg in kidney; 0.01, 0.02, 0.053, and 0.15 mg/kg in muscle; and 0.021, 0.024, 0.13, and 0.35 mg/kg in fat for each treatment group, respectively. At 48 h, the mean concentrations of total residues were 0.82 mg/kg in liver, 0.64 mg/kg in kidney, 0.091 mg/kg in muscle, and 0.097 mg/kg in fat in animals given lincomycin at 10-12 mg/kg of body weight. At 12 h, the mean concentrations of microbiologically active residues in tissues of animals given 10-12 mg/kg of body weight were 0.096 mg/kg in liver and 0.42 mg/kg in kidney. The samples of liver were reanalysed with an improved microbiological assay and by GC/MS. In liver samples from the group given the highest dose, the ratio of total residues to lincomycin was 17:1 at 12 h and 40:1 at 48 h after treatment.

In a study conducted using GLP conditions, twelve pigs received intramuscular doses of 11 mg/kg of body weight of ^{14}C-lincomycin daily for 3 days, and groups of three pigs were killed at 12 and 24 h and six pigs at 48 h after treatment (Nappier et al., 1988). Of the administered radiolabel, 78-85% was accounted for. The highest mean concentrations of total residue at 12 h were 17 mg/kg in liver and 12 mg/kg in kidney. The concentrations in other tissues were 1.0 mg/kg at the injection site, 0.59 mg/kg in fat, and 0.39 mg/kg in muscle. By 48 h, these concentrations had declined to 3.8 mg/kg in liver, 3.1 mg/kg in kidney, 0.58 mg/kg at the injection site, 0.20 mg/kg in fat, and 0.14 mg/kg in muscle. When the liver and kidney samples were reanalysed by an improved microbiological assay and by GC/MS, the ratio of total residues to lincomycin was 7.2 :1 at 12 h, 31 :1 at 24 h, and 62 :1 at 48 h in liver, and 1.8 :1 at 12 h, 5.1 :1 at 24 h, and 15 :1 at 48 h in kidney. Results are summarized in table 2.

Table 2. Mean ^{14}C-lincomycin total residue concentrations (mg/kg) in pig tissues after 11 mg/kg BW/day intramuscularly for three days.

Hours after treatment	Liver	Kidney	Muscle	Inj. Site	Fat
12	17.54 ± 6.24	12.02 ± 4.25	0.39 ± 0.04	1.05 ± 0.32	0.59 ± 0.36
24	13.61 ± 1.87	5.75 ± 0.24	0.13 ± 0.02	0.86 ± 0.28	0.26 ± 0.14
48	3.84 ± 1.41	3.08 ± 1.09	0.14 ± 0.04	0.58 ± 0.33	0.20 ± 0.07

Chicken

In a study conducted according to GLP, ^{14}C-lincomycin was given to 21 female and 21 male broiler chickens, 35 days old, in drinking-water at doses of 5.1-6.6 mg/kg of body weight per day for 7 days (Hornisch et al., 1984). Total radiolabelled residue was measured in liver, kidney, muscle, and skin with adhering fat immediately after withdrawal of the treated water and 0.5, 1, 2, 4, and 7 days after treatment. Liver and kidney contained the highest concentrations, with residues decreasing between day 0 and day 7 from 1.6 to 0.02 mg/kg in liver and from 1.3 to 0.01 mg/kg in kidney. The concentrations in muscle decreased from 0.052 at 0 h to <0.005 mg/kg at day 2, and in skin/fat from 0.132 at 0 h to <0.005 mg/kg at day 7. Lincomycin represented 20% of the radiolabel in liver at 0 h, 12% at 12 h, 8% at 24 h, 2% at 48 h, and 5% at 96 h. It represented 16% in muscle and 18% in skin with adhering fat immediately after the last treatment.

Table 3. Mean ^{14}C-lincomycin total residue concentrations (mg/kg) in chicken tissues treatment in drinking water for 7 days.

Days after treatment	Liver	Kidney	Muscle	Skin/Fat
0	1.58	1.26	0.052	0.013
0.5	0.503	0.56	0.027	0.051
1	0.224	0.23	0.027	0.065
2	0.107	0.10	<0.005	0.028
4	0.028	0.03	<0.005	0.017
7	0.020	0.01	<0.005	<0.005

In a study conducted according to GLP, eighteen laying hens were given capsules containing of ^{14}C-lincomycin, equivalent to 0.5 mg/kg of body weight per day, twice daily for 12 days (Gosline, Hornish 1980). Eggs were collected on days 1-3 of treatment, and tissues were collected from six animals 4, 28, and 76 h after treatment. The mean concentrations of total residue in eggs rose from 0.002 mg/kg at day 1 to 0.008 mg/kg at day 10 during treatment and had decreased to 0.005 mg/kg by day 2 after treatment. The mean concentrations of total residues were highest in kidney (0.15 mg/kg) and liver (0.14 mg/kg) and were lower in muscle (0.02 mg/kg) and skin with fat (0.02 mg/kg) 4 h after treatment. The residues in all tissues were depleted to < 0.01 mg/kg within 76 h. Results are summarized in table 4, while table 5 describes residues in eggs.

Table 4. Mean ^{14}C-lincomycin total residue concentrations (µg/kg) in laying hens after treatment for 12 days.

Hours after treatment	Liver	Kidney	Muscle	Skin & fat
4	141 ± 60.2	152 ± 94.6	19.7 ± 12.4	18.9 ± 4.4
28	24.3 ± 11.6	20.6 ± 5.5	12.7 ± 11.5	14.0± 7.3
76	5.7 ± 64	6.0 ± 6.8	9.8 ± 3.8	2.9 ± 7.2

Table 5. ^{14}C-Lincomycin total residue concentrations (μg/kg) in chicken eggs after treatment for 12 days.

	Days on treatment										Days after treatment		
Animal No	1	2	3	4	5	6	7	8	10	12	1	2	3
101	2.9	2.9	–	5.2	5.7	–	6.2	8.0	*	–	7.8	5.9	K-3
102	1.7	2.5	–	3.4	5	5.7	5.7	7.1	–	–	K-1		
103	1.3	NS	2.1	–	3.1	4.9	5.3	6.2	6.9	6.2	6.1	5.2	K-3
104	1.9	NS	2.9	3.2	3.7	4.4	5.0	5.5	5.9	5.1	–	K-2	
105	1.4	4.2	4.9	6.1	7.0	*	*	*	8.0	–	K-1		
106	2.3	3.2	–	6.9	7.4	8.0	–	9.0	12	–	9.8	K-2	
107	1.4	NS	–	2.4	3.4	4.1	4.3	–	6.9	4.7	–	4.1	K-3
108	1.4	NS	2.6	3.4	4.0	4.4	5.2	–	9.1	–	K-1		
109	–	–	3.3	4.3	4.9	6.0	5.9	6.5	7.3	6.7	5.9	K-2	
110	2.0	2.8	3.6	4.5	5	6.0	5.8	6.7	9.3	–	7.6	K-2	
111	1.2	NS	2.8	3.8	4.7	4.2	4.4	4.8	6.4	–	5.3	4.3	4.0, K-3
112	1.6	2.4	3.0	3.8	4.9	5.3	5.3	6.5	7.6	6.8	K-1		
113	1.7	3.5	–	4.9	6.5	–	7.8	–	–	10	–	K-2	
114	1.5	2.8	4.5	–	–	7.0	–	6.7	9.6	–	K-1		
115	2.0	NS	–	2.6	*	5.2	4.8	7.7	7.7	–	9.0	K-2	
116	1.4	2.6	2.4	2.9	–	4.1	4.8	–	6.6	5.0	5.1	4	K-3
117	–	3.2	4.5	5.7	8.6	7.5	7.9	–	11.9	*	9.2	–	K-3
118	2	*	3.6	–	5.0	6.3	*	7.9	–	*	K-1		
n	16	10	12	15	15	15	14	12	14	7	9	5	1
Mean	**1.7**	**3.0**	**3.4**	**4.2**	**5.3**	**5.5**	**5.6**	**6.9**	**8.2**	**6.4**	**7.4**	**4.7**	**4.0**
SD	0.4	0.5	0.9	1.3	1.5	1.3	1.1	1.2	1.9	1.8	1.7	0.8	–

Note: NS means not sampled.

Residue Depletion Studies with Unlabelled Drug

Lactating cows

In a study conducted prior to GLP, five lactating cows received three consecutive doses of 200 mg of lincomycin on one quarter of the udder at 12-h intervals (Barbiers et al. 1971). Milk samples were taken during treatment and at 12-h intervals for the following 10 milkings and analysed by microbiological assay (LOQ, 0.2 mg/kg). The mean concentrations of residue in milk decreased from 115 mg/kg at 12 h to 18 mg/kg at 24 h and 1.4 mg/kg at 36 h to below the LOQ at 48 h.

Three studies conducted according to GLP were reported of intramammary application of lincomycin.

The study in which the highest recommended dose was used involved 24 cows that received three consecutive intramammary infusions of 330 mg of lincomycin per quarter into each of the four quarters of the udder at 12-h intervals (Deluyker et al., 1996a. 1996b). Pooled milk samples were taken at 12-h intervals at eight milkings after the last application and analysed by GC/MS. The mean concentrations of lincomycin were 53 mg/kg at 12 h, 7.0 mg/kg at 24 h, <LOQ-4.0 mg/kg at 36 h, 0.02-1.5 mg/kg at 48 h, <LOQ-0.20 mg/kg at 60 h, and <LOQ (0.015 mg/kg) at all other times. Individual animal results are indicated in table 6.

Table 6. Lincomycin residue concentrations (mg/kg) in milk after 3 intramammary infusions of 330 mg/quarter/12 hour interval.

	Hours after treatment							
Cow No.	12	24	36	48	60	72	84	96
4	22.8	8.23	0.20	0.06	0.06	0.01	<LOQ	<LOQ
9	30.3	8.10	0.68	0.28	0.04	0.01	<LOQ	<LOQ
11	65.0	11.4	0.76	0.19	0.02	<LOQ	<LOQ	<LOQ
16	26.3	2.49	0.74	0.03	0.03	<LOQ	<LOQ	<LOQ
18	70.1	5.89	0.10	0.06	0.01	<LOQ	<LOQ	<LOQ
22	72.6	9.20	0.26	0.12	0.01	<LOQ	<LOQ	<LOQ
27	37.0	2.67	<LOQ	0.08	<LOQ	<LOQ	<LOQ	<LOQ
28	59.1	3.44	0.33	0.04	<LOQ	<LOQ	<LOQ	<LOQ
31	71.4	9.40	0.22	0.18	0.04	NA	NA	NA
37	43.0	5.10	0.72	0.14	0.06	<LOQ	<LOQ	<LOQ
40	34.4	1.95	0.55	0.06	<LOQ	<LOQ	<LOQ	<LOQ
41	58.0	6.75	0.22	0.08	0.01	<LOQ	<LOQ	<LOQ
46	53.8	5.00	0.35	0.14	0.02	NA	NA	NA
47	68.7	13.8	4.01	1.47	0.20	0.09	0.016	<LOQ
48	25.5	1.83	1.65	0.02	<LOQ	<LOQ	<LOQ	<LOQ
50	46.2	6.37	0.08	0.12	0.01	<LOQ	<LOQ	<LOQ
53	64.5	4.53	0.38	0.07	<LOQ	<LOQ	<LOQ	NA
57	61.7	9.60	0.31	0.24	0.02	<LOQ	<LOQ	<LOQ
60	73.2	10.4	0.84	0.24	0.05	<LOQ	<LOQ	<LOQ
61	57.4	7.67	0.91	0.26	0.07	<LOQ	<LOQ	<LOQ
63	77.2	8.25	0.88	0.24	0.04	<LOQ	<LOQ	<LOQ
65	36.5	6.14	0.91	0.24	0.03	<LOQ	<LOQ	<LOQ
71	70.7	14.0	0.75	0.38	0.16	<LOQ	<LOQ	<LOQ
73	49.5	5.23	0.46	0.07	0.03	<LOQ	NA	NA
Mean*	**53.1**	**6.98**	**0.68**	**0.20**	**0.04**			
SD*	17.4	3.44	0.80	0.29	0.05			
Min.	22.8	1.83	<LOQ	0.02	<LOQ			
Max.	77.2	14.0	4.01	1.47	0.20			

Note: NA is not analysed

Ten healthy cows received 3 consecutive doses of 197 mg lincomycin/quarter in two heterolateral quarters (right front and left rear) in 12 hour intervals (Nouws et al., 1994). Quarter and pooled milk samples were taken during treatment and at the 12 milkings following the last treatment and analysed using a bioassay (LOQ = 0.013 mg/kg). During treatment lincomycin concentrations in treated quarters were 36.1, 27.8 and 52.8 mg/kg declining to or below 0.07 mg/kg at 58 hrs and below 0.025 mg/kg at 72 hours.

Twelve healthy cows received 3 consecutive doses of 200 mg lincomycin/quarter (in two heterolateral quarters (n = 6), and right hind quarter, (n = 3), and left front quarter (n = 3) in 12 hour intervals (Deluyker et al, 1996b). Quarter and pooled milk samples were taken during treatment and at the 4 milkings following the last treatment and analysed using a bioassay (LOQ = 0.025 mg/kg). Mean residue concentrations during treatment were 36.7, 41.4 and 42.0 mg/kg in the three treatment groups, respectively, declining to 2.17 mg/kg at 24 h, 0.60 mg/kg at 36 h and 0.13 mg/kg at 48 hours.

In a 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-h intervals (DeGraeve et al, 1997). Tissue samples from four cows sacrificed at 1, 7, 14, and 21 days after treatment were analysed by GC/MS. The mean concentrations of lincomycin in liver were 0.23 mg/kg on day 1 and 0.058 mg/kg on day 7, and the ranges of concentrations of residues in liver were 0.017-0.040 mg/kg on day 14 and 0.007-0.051 mg/kg on day 21. Residues were found in muscle and kidney only at day 1, and no residues were found in fat.

Veal calves

In a study conducted according to GLP, four groups of five veal calves weighing 60-80 kg received daily intramuscular injections at different sites on both sites of the neck of 5 mg of lincomycin for 5 days. Two doses were given on the first day (Hoffman et al., 1996). The animals were killed and tissue samples were taken 8 h and days 7, 14, and 21 after treatment and analysed by GC/MS. At 8 h, the highest mean concentrations of residues were found in kidney (3.3 mg/kg) and at the last injection site (2.4 mg/kg). The mean concentration of residues in muscle was 0.72 mg/kg. The

concentrations were <LOQ-0.14 mg/kg in liver and <LOQ-0.26 mg/kg in fat at 8 h. The only other sample in which residues were detected was one of liver at day 14 (0.07 mg/kg).

Pig

Several residue depletion studies involving oral or intramuscular application of lincomycin to pigs were submitted.

The most relevant study conducted according to GLP, in which the maximal intramuscular dose was applied, involved two groups of 24 pigs which were given intramuscular doses of 11 mg/kg of body weight of two different formulations of licomycin for 3 days (Nappier et al, 1996d). The animals were killed 0, 3, 6, 12, 24, 48, and 144 h after treatment, and samples of liver, kidney, muscle, fat ,and injection site tissue were taken from four pigs in each group and analysed by GC/MS. The mean residue concentrations animals treated with the two formulations decreased rapidly between 3 and 48 h, from 6.4 and 4.7 mg/kg to 0.059 and 0.065 mg/kg in liver and from 29 and 21 mg/kg to 0.17 and 0.24 mg/kg in kidney. In both tissues, the concentrations were <LOQ at 144 h. In muscle, the mean concentrations were 3.6 and 2.6 mg/kg at 3 h, 0.061 and 0.085 mg/kg at 24 h, and <LOQ at all other times. In fat, the mean concentrations were 0.47 and 0.47 mg/kg at 3 h, 0.024 and 0.033 mg/kg at 24 h, and <LOQ at all other times. At the injection site, the mean concentrations were 115 and 250 mg/kg at 3 h, 0.022 and 0.025 mg/kg at 48 h, and <LOQ at 144 h. The results are summarised in table 7.

Table 7. Mean lincomycin residue concentrations (mg/kg) in pigs after intramuscular treatment for 3 days.

Preparation	Hours after treatment	Liver (n = 4)	Kidney (n = 4)	Muscle (n = 4)	Fat (n = 4)	Injection Site (n = 4)
Lincomix ® 100	3	6.37 ± 1.58	29.3 ± 5.67	3.60 ± 0.41	0.47 ± 0.30	115 ± 75
Lincomix ® 300	3	4.70 ± 0.61	21.0 ± 5.96	2.46 ± 0.42	0.47 ± 0.26	250 ± 68
Lincomix ® 100	6	4.36 ± 1.12	16.0 ± 1.77	2.31 ± 0.09	0.67 ± 0.20	15.1 ± 11.6
Lincomix ® 300	6	4.86 ± 1.33	18.4 ± 0.99	1.84 ± 0.53	0.46 ± 0.26	47.6 ± 44.1
Lincomix ® 100	12	2.42 ± 0.73	5.83 ± 1.45	0.69 ± 0.24	0.15 ± 0.14	2.55 ± 0.73
Lincomix ® 300	12	2.46 ± 0.65	7.47 ± 2.28	0.64 ± 0.10	0.20 ± 0.12	7.12 ± 4.20
Lincomix ® 100	24	0.32 ± 0.14	0.91 ± 0.15	0.06 ± 0.02	0.02 ± 0.01 1 <LOQ	0.23 ± 0.14
Lincomix ® 300	24	0.55 ± 0.32	1.36 ± 0.45	0.08 ± 0.02	0.04 ± 0.03 1 <LOQ	1.27 ± 0.50
Lincomix ® 100	48	0.06 ± 0.02	0.17 ± 0.09	2 of 4 <LOD	4 <LOQ 1 <LOD	0.02 ± 0.01 1 <LOQ
Lincomix ® 300	48	0.06 ± 0.02	0.24 ± 0.17	0.01 ± 0.00	4 <LOQ 1 < LOD	0.02 ± 0.01 1 <LOQ
Lincomix ® 100	144	<LOQ	<LOQ	3 <LOD	4 <LOD	0.003 ± 0.00
Lincomix ® 300	144	<LOQ	<LOQ	3 <LOD	4 <LOD 3 <LOD	0.031 ± 0.05 3 <LOQ

Sheep

In a study conducted according to GLP, four groups of five sheep received daily intramuscular doses of lincomycin at 5 mg/kg of body weight (formulated with 10 mg/kg spectinomycin) for 3 days (Brown et al, 1996a, 1996b). The animals were killed 8 h and days 7, 14, and 21 after treatment, and liver, kidney, muscle, and injection site tissue were analysed by GC/MS. At 8 h after the last treatment, the mean concentrations of lincomycin were highest in kidney (9.0 mg/kg), liver (4.3 mg/kg), and at the site of injection (14 mg/kg); a lower mean concentration (0.95 mg/kg) was found in muscle. By day 7, only two of five samples of liver contained concentrations of residues that were above the LOQ of the method.

Chicken

Chicken broilers were treated orally via the drinking water with lincomycin /spectinomycin of doses of 1 g + 2 g/gallon for 7 days (Barbiers, Smith 1968). Tissue samples were taken at 0 (n = 2), 6, 12, 18 (n = 4 each) for liver, muscle and fat/skin, (n = 2) for kidney at 24 h and at 48 h (n = 4 each) for liver, muscle and fat/skin, (n = 2) for kidney. Samples were analysed by bio-assay. Lincomycin was detected at 0 h in one liver sample at 0.98 mg/kg and at 6 h in one kidney sample at 0.85 mg/kg. Respective serum samples showed only positive results at 0 h (0.18) and at 12 h (0.34) mg/kg.

EFFECT OF LINCOMYCIN ON STARTER CULTURES IN MILK PROCESSING

The effect of lincomycin on bacterial starter cultures was investigated in cultures used for the production of Italian cheese, yoghurt, butter milk and sour cream. For each starter culture, the four- parameter Weibull growth curve was used to model the pH as a function of time. At concentrations up to 0.16 mg/kg no significant effects were observed.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUE AND MILK

Various methods have been used to determine the concentrations of residues of lincomycin in animal-derived foods. The methods include microbiological assay, thin-layer chromatography-bioautography, GC with an alkaline flame detector, and GC/MS.

Screening test for milk

In AOAC Official Method 988.08 (AOAC International, 1995), lincomycin is detected in milk samples based on a binding reaction between incurred residue and binding sites on a substrate. The method uses a commercially available test (Charm Test) validated at 0.40 mg/kg for the detection of lincomycin residues in milk.

Microbiological Assays

In one of the early reports of analytical methods for lincomycin residues in milk, *Sarcina lutea* ATCC 9341 was used as the test organism, seeded on a test plate containing Antibiotic Medium 11 (Barbiers and Gosline, 1973). The pH of the milk was adjusted to 8.5 with NaOH (3.5 and 1.0 M) and a sample was added to a steel sample cylinder in contact with the test medium. Following incubation for 18 hrs at 32°C, the size of inhibition zone was measured and compared with standards. The limit of detection (LOD) was claimed to be 0.1 mg/kg. Subsequently, the same organism was used in a microbiological plate assay was used for screening for lincomycin residues in tissue samples, as well as in TLC-bioautography for confirmation (Barbiers and Neff, 1976). The samples were cleaned up using a XAD resin, then chromatographed on silica gel TLC plates. The developed plate was dried, then brought into contact with growth medium for 30 min, after which the TLC plate was removed. Following overnight incubation, lincomycin was identified by the presence of a zone of inhibition at a location corresponding to the R_f of lincomycin on the TLC plate. The method was tested for liver, kidney, muscle and skin/fat of poultry, calf, lamb and pig, with a LOD of 0.1 mg/kg.

More recently, lincomycin was extracted from milk using a C-18 SPE cartridge and quantified using *M. luteus* as a test organism for agar diffusion test plates at pH 8 (Deluyker et al, 1996a). Recovery was 70%, with a limit of quantitation (LOQ) of 0.025mg/kg, based on 6 replicate spiked samples at this level, and a LOD of 0.013 mg/kg. The analytical range tested was 0.025-0.80 mg/kg.

Chemical Methods

A method was reported in which regulatory samples of bovine and porcine kidney were analyzed by gas chromatography with nitrogen specific detection after an extensive clean-up which included an initial clean-up using a solid phase extraction cartridge, collection of a fraction from a high performance liquid chromatography separation, followed by solid phase extraction of the collected fraction (Farrington et al, 1987). The final extract was derivatized with a silylating reagent and analyzed by gas chromatography with an alkali flame detector operated in the nitrogen-specific mode. Recovery from bovine and porcine kidneys spiked at 0.1 mg/kg ranged between 31-51% and 38-64%, respectively. Four batches of each tissue were run on separate days, with 3 replicates per run. The LOD was estimated to be 0.02 mg/kg, with a linear analytical range from 0.02 to 0.20 mg/kg. The method was applied in a pilot survey to 54 samples. However, the length and complexity of the clean-up procedure would make this method rather difficult to apply on an on-going routine basis.

The method used by the sponsor in various residue depletion trials and proposed for regulatory use is based on a GC/MS procedure originally developed for lincomycin residues in human serum (Fourtillan et al, 1987). Following clean up by solid phase extraction and derivatization with acetic anhydride, lincomycin was detected as the m/z 126 fragment ion using GC/MS in the electron impact (EI) mode. The method was later validated for the analysis including pig fat, liver, kidney and muscle, chicken fat, liver, kidney, muscle and skin, and calf liver (Mignot et al, 1991).

The method was also adapted and applied to the analysis of pig liver, with the introduction of an acidic extraction step (Nappier and Hoffman, 1989). A correlation of >0.98 was obtained when the results of the GC/MS and microbiological assays were compared. In a later study, it was shown that the original method for lincomycin in serum could also be successfully applied to pig serum using an ion trap mass spectrometer, rather than a conventional quadrupole mass spectrometer (Hoffmann et al, 1990). Additional work demonstrated that the GC/MS and microbiological assays for lincomycin in pig plasma gave comparable results (Davis and Hamlow, 1990).

Subsequently, it was discovered during sample storage stability studies with freeze-thaw cycles that the recovery of incurred lincomycin residues from pig liver increased by over 2-fold when the sample was incubated at room temperature overnight (Nappier and Rizzo, 1995; Nappier et al, 1996b). Further study demonstrated that the analysis of pig kidney and muscle, as well as sheep liver and kidney, did not require the 18 h incubation required for pig liver to release residues (Nappier et al, 1996c; DeYoung et al, 1996a). Results obtained with and without the incubation step for these tissues agreed within 15%.

The original tissue version of the method was modified to require a smaller sample size, to include the incubation step for pig liver and to provide requirements for confirmatory analysis (Nappier et al, 1995). After an extensive sample clean-up, a 1µl aliquot is injected into the GC/MS at an oven temperature of 175°C, held for 0.5 min., then ramped at 30 °C/min to 325 °C and held there for 5 min. Total run time is 10 min, with injection port and transfer line temperatures at 300 °C and helium flow rate of 1 mL/min. A 30m x 0.25mm id containing a cross-linked methyl silicone coating, 0.33 µm film thickness, was used for the analysis, in combination with a 5m x 0.25mm id pre-column. Assay precision was similar for analysis of duplicates of both fortified and incurred samples (11.2-12.1% from 0.078 to 0.321mg/kg for liver and 9.6-11.9% from 0.115 to 0.616 mg/kg for kidney). The confirmatory procedure for residues in pig liver uses the molecular ion fragment m/z 575 and three fragments at m/z 126, 515 and 527 in chemical ionization mode, using methane as the reactant gas. Confirmation required a retention time within 2% of the mean standard retention times, relative ion intensities within 10% of the mean standard relative ion intensities and a signal-to-noise ratio of >3 for at least 3 ions.

In a method comparison, 19 pig kidney and 17 pig liver samples previously assayed by GC/MS were also analysed by the cylinder plate assay method, with results from the two methods correlated at 0.95 for both liver and kidney analyses (Nappier et al, 1996f).

The determinative method was also validated for sheep liver (Gammill et al, 1995), sheep kidney (DeYoung et al, 1995), sheep muscle (DeYoung et al, 1996b), chicken liver (DeYoung et al, 1997a), chicken kidney (DeYoung et al, 1997b), chicken gizzards (DeYoung et al, 1997c) and chicken muscle (DeYoung et al, 1997d). In these more recent studies, the capillary column used in the GC/MS analysis is a 12.5m x 0.20mm id column coated with an 0.33 µm film thickness of cross-linked methyl silicone.

The method was modified for the analysis of fat by including an initial extraction of the 3 g analytical sample with chloroform: hexane (DeYoung et al, 1996c,d) and also applied to the analysis of chicken fat (DeYoung et al, 1997e). The modification was also shown to be necessary for the analysis of bovine fat, but bovine liver, kidney, muscle and milk could be analyzed without the inclusion of the initial organic extraction (Nappier et al, 1997a). A method has also been tested for the analysis of lincomycin in eggs, but a full validation report was not available (Nappier to Thomas, 1998). The results of the validation tests for various matrices have been summarised in Table 8 (Nappier, 1998).

Table 8. Summary of validation study results for analysis of lincomycin residues by electron impact gas chromatography – mass spectrometry (GC/MS-EI) in various edible tissues and milk.

Species	Edible Tissue or Milk	Limit of Detection (mg/kg)	Limit of Quantitation (mg/kg)	Mean Recovery (%)	Repeatability (%)	Reproducibility (%)
Bovine	Milk	0.008	0.015	102	2.9	9.6
	Muscle	0.015	0.015	90	5.0	11.2
	Liver	0.005	0.015	93	8.0	9.9
	Kidney	0.007	0.015	96	5.5	12.7
	Fat	0.005	0.015	97	5.9	6.9
Pig	Muscle	0.002	0.017	96	3.8	4.8
	Liver	0.03	0.06	97	5.7*	11.7
	Kidney	0.03	0.06	91	5.2	12.0
	Fat	0.005	0.017	85	7.1*	10.0
Chicken	Muscle	0.003	0.017	85	5.8*	8.2
	Liver	0.003	0.017	92	5.6*	9.1
	Kidney	0.003	0.017	85	5.5*	9.4
	Fat	0.002	0.017	93	7.3*	9.9
Sheep	Muscle	0.002	0.017	93	3.1*	4.1
	Liver	0.003	0.017	84	5.2*	6.7
	Kidney	0.002	0.017	85	2.8*	5.0

*Based on analysis of variance techniques applied to data from referenced technical reports.

MAXIMUM RESIDUE LIMITS

In recommending MRLs, the Committee took into account the following:

- An ADI of 0-0.03 µg/kg of body weight was recommended by the Committee on the basis of a microbiological end-point, which results in a maximum daily intake of 1.8 mg for a 60-kg person.
- In pig tissues, lincomycin is the major component with significant microbiological activity.
- In milk, lincomycin accounts for 90% of the total residues.
- There was insufficient evidence that lincomycin is the major component with significant microbiological activity in tissues of cattle, sheep, and chicken and in chicken eggs.
- Lincomycin is the marker residue.
- Kidney and liver contain the highest concentrations of residues.
- A validated GC/MS method is available which could be used routinely in many laboratories and has a LOQ of 0.02-0.06 mg/kg in tissues of pigs, cattle, calves, sheep, and chicken.
- 0.16 mg/kg is the concentration below which lincomycin has no effect on bacterial starter cultures in the production of milk products.
- Lincomycin was considered as a drug with a long history of use.

On the basis of the above considerations, the Committee recommended the following MRLs for lincomycin in edible tissues of pigs and in cows' milk, expressed as parent drug : muscle,0.1 mg/kg, liver,0.5 mg/kg, kidney.1.5 mg/kg, fat,0.1 mg/kg and milk of dairy cattle, 0.15 mg/kg. Considering its old drug policy, the Committee extended the same MRLs as temporary for muscle, liver, kidney and fat in cattle, calves, sheep and chicken. The Committee was unable to recommend MRLs for chicken eggs.

Using the conservative consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, and 50 g of fat and 1.5 kg milk, the theoretical maximum intake of lincomycin residues would be 0.385 mg.

Before reviewing the compound again, the Committee would wish to receive the following information by 2002:

1. Comparable data as have been provided for tissues of pigs, which show that lincomycin is the major component with significant microbiological activity in tissues of cattle, calves, sheep, chicken and in chicken eggs.
2. Residue depletion study in chicken eggs using the GC/MS method.

REFERENCES

AOAC International. (1995) AOAC International Official Method 988.08: Antimicrobial Drugs in Milk. Microbial Receptor Assay. AOAC Official Methods of Analysis, 16th Edition, AOAC International, Gaithersburg, MD, Chapter 33, pp. 42-43.

Barbiers, A.R. and Gosline, R.E (1973). An assay for fincomycin in milk sensitive to 0. 1 ppm. Upjohn Interoffice Memo to AW Neff, 2 August 1973

Barbiers, A.R. and Kleckner, M.D. (1964) Lincomycin serum levels in pigs following intramuscular and oral administration of 2 and 5 mg/kgb. The Upjohn Company Interoffice Memo. 13 July 1964.

Barbiers, A.R. and Kleckner, M.D. (1964) Serum levels and tissue residues of lincomycin in pigs following treatment for three consecutive days, intramuscularly and orally. The Upjohn Company Interoffice Memo, 17 September 1964.

Barbiers, A.R., Krzeminski, L.F., Perrel, P.N., Schiltz, R.A., Bell, R.L., Lallinger, A.J., Cox, B.L., Smith, L.J. and Subacz, C.J. (1971) Persistence study in lactating dairy heifers following intramammary infusion with Lincocin Forte®. Upjohn Technical Report 712-9760-9, 9 December 1971.

Barbiers, A.R. and Neff, A.W. (1976) Screening and confirmatory methods for determining lincomycin residues in animal tissues. J. of the AOAC 1976; 59; 849-54.

Barbiers, A.R. and Smith, L.J. (1968) Lincomycin broiler tissue residues following oral spectinomycin/lincomycin via drinking water. Upjohn Technical Report 723-9760-13, 21 November 1968.

Barbiers, A.R., Smith, L.J., Swenson, G.H. and Lallinger, A.J. (1975) Distribution of lincomycin in tissues and body fluids in pig following intramuscular administration. Upjohn Technical Report 003-9760-8. 30 January 1975.

Brown, S.A., Johnson, R.A., Craigmill, A.L. and Wetzlich, S. (1996a) LINCO-SPECTIN™ Sterile Solution in Sheep. Part IV. Method validation and residue decline of lincomycin in sheep liver after multiple intramuscular administration at a dose of 15 mg/kg (5 mg lincomycin and 10 mg spectinomycin/kg). Upjohn Technical Report 803-7926-95-003, 12 January 1996.

Brown, S.A., Johnson, R.A., Craigmill, A.L. and Wetzlich, S. (1996b) LINCO-SPECTIN™ Sterile Solution in Sheep. Part V. Method validation and residue decline of lincomycin in sheep liver after multiple intramuscular administration at a dose of 15 mg/kg (5 mg lincomycin and 10 mg spectinomycin/kg). Upjohn Technical Report 803-7926-96-001, 14 February 1996.

Daniels, E.G. and VanEyk, R.L. (1978) Metabolism of lincomycin hydrochloride in dogs. Excretion of radioactivity and characterisation of urinary and faecal metabolites. Upjohn Technical Report 7261-78-7261-008. 1 November 1978.

Davis, W.M. and Balcolm III, J.H. (1969). Lincomycin studies on drug absorption and efficacy. Oral surgery,1969. 27 (5): 688 -96.

Davis, R.A. and Hamlow, P.J. (1990). Comparison of microbiological and instrumental assays for the determination of lincomycin and spectinomycin in pig plasma. Research Report 803-9760-90-002, Sponsor Submitted.

DeGeeter, M.J. and Barbiers, A.R. (1978) Lincomycin residues in tissue and serum from pig fed diets fortified with O or 44 mg lincomycin per kilogram. Upjohn Technical Report 524-78-9660-016. 19 October 1978.

DeGraeve, J., Van Heugen, I-C, Nappier, J.L. and Deluyker, R.A. (1997) Tissue residue depletion study of UNCOCIN® FORTE Sterile following intramammary infusions to dairy cows. Part II - lincomycin assay validation, and lincomycin residues. Pharmacia & Upjohn Technical Report 804-7926-97-00 1, 11 February 1997.

Deluyker, H.A., Nouws, J.F.M., Van Oye, S. and Gilberston, T.J. (1996a) Concentrations of neomycin and lincomycin in milk during and following three successive intramammary infusions of Lincocin Forte ® in normal lactating cows. Upjohn Technical Report 804-7926-95-001, 23. January 1996.

Deluyker, H.A., VanHeugen, J.C., DeGraeve, J. and Nappier, J.L. (1996b) Tolerance, kinetics, and milk residue depletion study of LJNCOCIN FORTE™ Sterile following intramammary infusions to dairy cows - Part II Pharmacia & Upjohn Technical Report 804-7926-96005, 18 December 1996.

DeYoung, L.A., Grostic, M.F., and Johnson, R.A. (1995). Method Validation Report: GC/MS assay for lincomycin in sheep kidney tissue. Research Report 95-550, Sponsor Submitted.

DeYoung, L.A., Johnson, R.A., Nappier, J.L., and Brown, S.A. (1996a). Method validation report addendum: tissue incubation and parallelism results. Research Report 803-7926-96-003, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A., Nappier, J.L. (1996b). Method validation report: GC/MS-EI assay for lincomycin in sheep muscle tissue. Research Report 768-7926-96-002, Sponsor Submitted.

DeYoung, L.A., Gammill, J.C., Johnson, R.A., Johnson, K.M., and Grostic, M.F. (1996c). GC/MS assay for lincomycin free base in pig fat tissue. Research Report 96-670, Sponsor Submitted.

DeYoung, L.A., Johnson, R.A., Nappier, J.L. and Rizzo, V.L. (1996d). Method validation report: GC/MS-EI assay for lincomycin in pig fat tissue. Research Report 768-7926-96-009, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997a). Method validation report: GC/MS-EI assay for lincomycin in chicken liver tissue. Research Report 768-7926-97-002, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997b). Method validation report: GC/MS-EI assay for lincomycin in chicken kidney tissue. Research Report 768-7926-97-003, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997c). Method validation report: GC/MS-EI assay for lincomycin in chicken gizzard tissue. Research Report 768-7926-97-004, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997d). Method validation report: GC/MS-EI assay for lincomycin in chicken muscle tissue. Research Report 768-7926-97-005, Sponsor Submitted.

DeYoung, L.A., Smith, A.R., Johnson, R.A. and Nappier, J.L. (1997e). Method validation report: GC/MS-EI assay for lincomycin in chicken fat tissue. Research Report 768-7926-97-008, Sponsor Submitted.

Eberts, F.S., Meeks, R.C. and Vliek, R.W. (1963) Status report: Metabolism of ^{3}H-Lincomycin in the dog. The Upjohn Company Interoffice Memo, 17. 26 September 1963.

Farrington, W.H.H., Cass, S.D., Patey, A.L. and Shearer, G. (1987) A method for the analysis of lincomycin in porcine and bovine kidney. Food Add. and Contam. 1: 67-76.

Fourtillan, J.B., Mignot, A., Lefebvre, M.A., Girault, J., Parsy, P., Senon, J., and Ingrand, I. (1987) Assay of lincomycin-A in plasma samples by combined gas chromatography – electron impact mass spectrometry. Research Report CE572H, Sponsor Submitted.

Gammill, J.C., DeYoung, L.A., Grostic, M.F. and Johnson, R.A. (1995) Method Validation Report: GC/MS assay for lincomycin in sheep liver tissue. Research Report 95-520, Sponsor Submitted.

Gosline, R.E., Barbiers, A.R., Subacz, C.J. and Smith, L.J. (1978) ^{14}C-Lincomycin (U-10, 149A) fate study in chickens. Upjohn Technical Report 768-78-9760-001, 26 April 1978.

Gosline, R.E. and Hornish, R.E. (1980). The disposition of ^{14}C-Lincomycin HCI (U-10,149A) in laying hens. Upjohn Technical Report 536-9760-80-001, 29 April 1980.

Hoffman, G.A., Nappier, J.L., Hanson, B.J., and Gilbertson, T.J. (1990). Assay validation of a gas chromatography/mass spectrometry – ion trap detection procedure for the determination of lincomycin (U-10,149A) as the anhydrous free base in the plasma of pig. Research Report 768-9760-90-002, Sponsor Submitted.

Hoffman, G.A., Delahaut, P., DeGraeve, 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.

Hornish, R.E., Arnold, T.S., Cox, T.D., Hubbard, V.I., Nappier, J.M., Pierce, P.A., Reeves, D.R., Stahl, G.I., Subacz, C.J. and Zaya, M.J. (1987) Lincomycin residues in the tissues of pig treated with ^{14}C-lincomycin hydrochloride by feed additive at 20,40, 100, and 200 grams per ton. Upjohn Technical Report 768-9760-87-001, 6 August 1987.

Hornish, R.E. and Gosline, R.E. (1981) Metabolism of ^{14}C-lincomycin fed at 200 g/ton in pig. Part I - Accountability, distribution and depletion of ^{14}C in tissue. Upjohn Technical Report 768-9760-81-001. 27 January 1981.

Hornish, R.E., Gosline, R.E., Nappier, J.M., Butine, T.J. and Subacz, C.J. (1984) The metabolism and depletion of 14C-Lincomycin (U-10,149A) in broiler chickens exposed at 128 mg/gal in the drinking water. Part I. Upjohn Technical Report 783-9760-84-001, 14 February 1984.

Hornish, R.E., Gosline, R.E., Arnold, T.S., Butine, T.J., Smith, L.J. and Subacz, C.J. (1985) Pharmacokinectics and oral absorption of lincomycin (U-10, 149) in pig. Upjohn Technical Report 002-9760-85-002. 25 September 1985.

Hornish, R.E., Gosline, R.E. and Nappier, J.M. (1987) Comparative metabolism of lincomycin in the Pig, Chicken and Rat. Drug Metabolism Reviews, 1987. 18 (2&3): 177-214.

Kaplan K, Chew, W.H. and Weinstein, L. (1965) Microbiological pharmacological and clinical studies of lincomycin. Am. J. of Med. Sci., 1965, 250:137-146.

Mignot, A., LeFebvre, M.A., Parsy, P., Renoux, A. and Gilbertson, T.J. (1991). Validation of a GC/MS assay for lincomycin with limit of detection of 0.04 mg/kg. Research Report 768-7926-91-001, Sponsor Submitted.

Nappier, J.L. and Hoffman, G.A. (1989) The determination of lincomycin in the liver of intramuscularly and orally treated pig by GC/MS analysis. Research Report 768-9760-89-001, Sponsor Submitted.

Nappier, J.L., Hoffman, G.A., Arnold, T.S., Cox, T.D., Reeves, D.R., Flook, T.F. and Paulissen, J.B. (1988). Total residues of ^{14}C-lincomycin in the tissues of intramuscularly treated pig. Upjohn Technical Report 768-9760-88-001, 8 September 1988.

Nappier, J.L., Hoffman, G.A., Yein, F.S., Zaya, M.J. and Paulissen, J.B. (1989) The determination of lincomycin in the liver of intramuscularly and orally treated pig by a modified GC/MS procedure: Validation, correlation and analysis. Pharmacia and Upjohn Technical Report 768-9760-89-002, 23 June 1989.

Nappier, J.L. and Rizzo, V.L. (1995) Determination of the appropriate conditions for the improved extraction of lincomycin from treated swine liver. Research Report 768-9760-89-001. Sponsor submitted.

Nappier, J.L., Rizzo, V.L., Arnold, T.S., Janose, R.l., Cox, T.D., Flook, T.F., Lewis, V.R., and Hanson, B.J. (1995). Validation of two methods of analysis of lincomycin hydrochloride residue in pig by GC/MS. A determinative method in pig liver and kidney and a confirmatory method in pig liver. Research Report 768-7926-95-001, Sponsor Submitted.

Nappier, J.L., Hanson, B.J., DeYoung, L.A. and Johnson, R.A. (1996a) The determination of the residue decline of lincomycin in the muscle tissue of pig treated with an intramuscular injection of lincomycin hydrochloride (U-10149A) at 11 mg of lincomycin free base equivalents per kg of body weight. Pharmacia & Upjohn Technical Report 768-7926--96-004, 23 October 1996.

Nappier, J.L., Rizzo, V.L., Fate, G.D., and Benner, C.P. (1996b). The discovery and resolution of the incomplete extraction of antibiotic residues from the liver of treated pig. Proceedings of the Euroresidue III Conference, Veldhoven, the Netherlands, pp. 740-744.

Nappier, J.L., Rizzo, V.L., Staskiewicz, S.G., DeYoung, L.A., and Johnson, R.A. (1996c). Evaluation of the need for incubation of pig and sheep tissues in the residue analysis of lincomycin. Research Report 768-7926-96-006, Sponsor Submitted.

Nappier, J.L., Rizzo, V.L., Staskiewicz, S.G., Stafford, L.K., Arnold, T.S., Janose, R.L., Lewis, V.R., Cox, T.D., Flook, T.F. and Hanson, B.J. (1996d) The determination of the residue decline of lincomycin in the liver and kidney tissues of pig treated with an intramuscular injection of lincomycin hydrochloride (U-10,149A) at 11 mg of lincomycin free base equivalents per kg of body weight. Upjohn Technical Report 768-7926-95-004, 13 February 1996.

Nappier, J.L., Rizzo, V.L., Staskiewicz, S.G., Stafford, L.K., Arnold, T.S., Janose, R.L., Lewis, V.R., Cox, T.D., Flook, T.F. and Hanson, B.J. (1996e) The determination of the residue decline of lincomycin in the liver and kidney tissues of pig treated with lincomycin hydrochloride (U-10,149A) at 44, 110, or 220 grams of lincomycin free base equivalents per 1000 kg of feed. Upjohn Technical Report 768-7926-95-003, 13 February 1996.

Nappier, J.L., Stafford, L.K.E., Rizzo, V.L., Staskiewicz, S.G., DeYoung, L.A., Johnson, R.A. and Fate. G.D. (1996f) Correlation of the lincomycin liver and kidney tissue residue assays: GC/MS assay vs microbiological assay. Pharmacia & Upjohn Technical Report 768-7926-96-007, 3 September 1996.

Nappier, J.L., DeGraeve, J, Van Heugen, J.C. and Deluyker, H.A. (1997a). Lincomycin bovine feasibility study: selection of an appropriate analytical assay for the determination of lincomycin residues in milk and animal tissue. Research Report 804-7926-97-002, Sponsor Submitted.

Nappier, J.L., Hanson, B.J., DeYoung, L.A. and Johnson, R.A. (1997b). The determination of the residue decline of lincomycin in the injection site tissues of pig treated with an intramuscular injection of lincomycin hydrochloride (U- 10 149A) at 11 mg of lincomycin free base equivalents per kg of body weight. Pharmacia & Upjohn Technical Report 768-7926-96-008, 12 May 1997.

Nappier, J.L., Hanson, B.J., DeYoung, L.A. and Johnson, R.A. (1997c) The determination of the residue decline of lincomycin in the fat tissue of pig treated with an intramuscular injection of lincomycin hydrochloride (U- 10149A) at I I mg of lincomycin free base equivalents per kg of body weight. Pharmacia & Upjohn Technical Report 768-7926-96-011, 13 May 1997.

Nappier, J.L. (1998). Additional clarification of lincomycin MRL residue file. Pharmacia & Upjohn Interoffice Memo, 15 June 1998.

Nappier, J.L. (1998). A summary of the GC/MS assay procedures for the analysis of lincomycin in biological matrices. Research Report 768-7926-97-001, Sponsor Submitted.

Nappier, J.L. to Thomas, R.W. (1998) Lincomycin GC/MS egg assay. Interoffice memo, August 25 1998, Sponsor Submitted.

Nappier, J.L., Brown, S.A., Craigmill, A.L., Wetlich, S. (1998) LINCO-SPECTIN Sterile Solution in sheep. Part VI. Residue decline in sheep muscle and injection site after multiple intramuscular administrations at a dose of 15 mg/kg (5 mg lincomycin and 10 mg spectinomycin/kg). Pharmacia & Upjohn Study Report aOO 18987, 5 October 1998.

Nouws, J.F.M., Deluyker, H.A., Gilbertson, T.J. and Chester, S.T. (1994) Concentration of neomycin and lincomycin during and following three successive intramammary infusions of Lincocin Forte ® in normal lactating dairy cows. Upjohn Technical Report 804-7926-94-001, 3 August 1994.

Russel, C.A. (1979) Production of serum time curve in the pig following a single intramuscular injection of Lincocin ® Sterile Solution (100 mg/mL). Upjohn Technical Report 777-0008-79-001. April 1979.

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

MELENGESTROL ACETATE

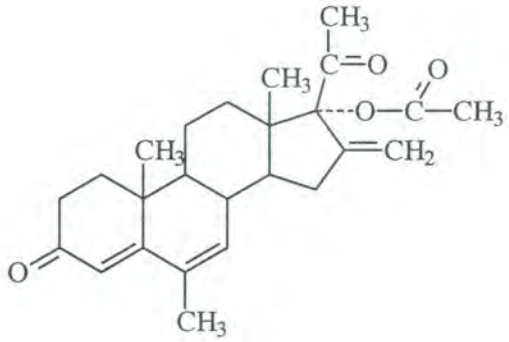
First draft prepared by
Dieter Arnold, Berlin, Germany
Rainer Stephany, Bilthoven, The Netherlands

IDENTITY

Chemical name: 17α-Acetoxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione acetate (Chemical Abstracts name); CAS No. 2919-66-6

International nonproprietary name: Melengestrol Acetate
Manufacturer's code, PNU-21240

Chemical structure:



Molecular formula: $C_{25}H_{32}O_4$

Molecular mass: 396.53

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White powder
Color: Off-white to light yellow
Melting point: 224-226 °C

Optical rotation: $[\alpha]_D^{23}$ -127° (c = 0.31 in chloroform)

Ultraviolet maximum: 287nm (in ethanol;log ε 4.35)
Solubility: 1.06 mg/L in water

RESIDUES IN FOODS AND THEIR EVALUATION

Conditions of use

Melengestrol acetate (MGA) is an orally active progestogen. It is used to improve feed conversion efficiency, promote growth and suppress estrus in female beef cattle fed for slaughter. The range of approved doses is 0.25 to 0.50 mg/heifer/day. Melengestrol acetate is fed for the duration of the fattening/finishing period, usually 90 to 150 days. Melengestrol acetate has not previously been evaluated by the Committee.

PHARMACOKINETICS AND METABOLISM

Studies with radio-labeled melengestrol acetate were carried out to determine its fate in feedlot heifers (**Krzeminsky *et al.*** 1981). Young Angus-Hereford heifers, housed in individual box stalls, received complete ground ration

supplemented with 0.5 mg of unlabelled melengestrol acetate/animal/day for four months. The animals were moved to metabolism stalls and acclimated. Three animals then received daily oral doses (in capsules) of approximately 100 µCi (equivalent to approximately 0.5 mg) of ^{3}H-melengestrol acetate, labeled in the 6-methyl group, for 21 days. One animal received daily oral doses (in capsules) of 254 µCi (equivalent to approximately 0.5 mg) of ^{14}C-melengestrol acetate, labeled in the 6-methyl group and in the 16-methylene group, for seven days. Radioactivity was determined in urine and faeces. Each heifer was slaughtered 6 h after the last capsule had been administered and total residues in body fluids and tissues were determined.

Significant losses of the ^{3}H - label (formation of ^{3}H-labeled water from the methyl group in 6-position) were observed in this study. The radioactivity was eliminated from the heifers via the faeces and urine in a 6:1 ratio. Independent studies of animals with bile cannulation showed that biliary excretion closely parallels total faecal output. In the present study, the highest concentration of total residue was found in liver; but the highest percentage of parent melengestrol acetate was found in fat. The concentrations found in visceral fat, omental fat and perirenal fat were similar. Whereas the results obtained for fat and liver were reproducible in all four treated animals, the concentrations found in muscle were variable because they were at or below the limit of detection of the radioactivity 6 h after the last treatment. Individual metabolites were not identified in the study because of their low concentrations. The individual results are given in Table 1.

Table 1. Total residue (µg/kg) and percent radio-labeled melengestrol acetate in tissues at steady state.

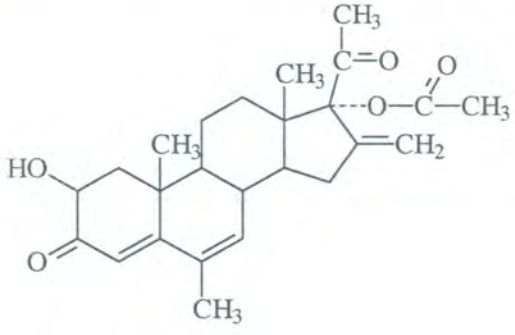
	Animal 1		Animal 2		Animal 3		Animal 4	
Tissue	Total residue	% ^{3}H-MGA	Total residue	% ^{3}H-MGA	Total residue	% ^{3}H-MGA	Total residue	% ^{14}C-MGA
Perirenal fat	7.5	78	7.7	86	8.0	94	3.6	75
Muscle	0.6	31	1.0	72	0.5	40	NS	45
Liver	12	30	15	30	9.0	28	8.2	37
Kidney	1.7	24	1.8	34	1.2	130	1.6	30

NS, Not significantly above background.
The limit of quantification is about 0.5 µg/kg in this study

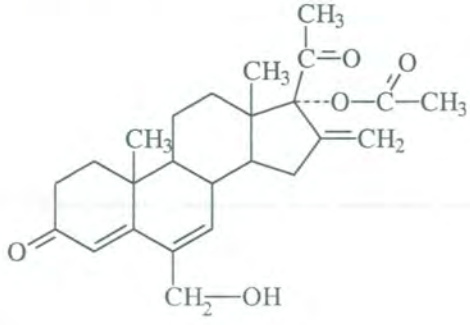
Under conditions of *in vitro* incubation of ^{3}H-melengestrol acetate with liver homogenates the 6-methyl- ^{3}H - label was reported to be stable (37°C, 3 hours). About 40% of radioactivity in the homogenate represented melengestrol acetate. The remainder of the radioactivity represented several unidentified degradation products (**Jaglan**, 1975a).

Incubation of ^{3}H-melengestrol acetate in fresh bovine rumen fluid *in vitro* for 24 and 96 hours at 37°C also resulted in no loss of ^{3}H - label from the molecule. All acetonitrile extractable radioactivity co-chromatographed with melengestrol acetate. However, when rumen fluid was collected from a heifer slaughtered six hours after the final dose of a 23-day treatment period with ^{3}H-melengestrol acetate, 28.5% of the recovered radioactivity was incorporated into water (**Jaglan**, 1975b).

In women melengestrol acetate was metabolized to at least thirteen distinct metabolites (**Cooper**, 1968). The only metabolites identified in experimental animals and in humans were the 2α-hydroxy- and 6-hydroxymethyl -metabolites, respectively, of the parent compound. 2α-hydroxy-melengestrol acetate was excreted in the urine of women and rabbits. 6-hydroxy-methyl -melengestrol-acetate was only identified in urine of rabbits.



2α-hydroxy-melengestrol acetate



6-hydroxymethyl-melengestrol acetate

When 4.0 mg of ^{3}H-melengestrol acetate was given orally as a single dose to a Holstein heifer, the radioactivity was quantitatively recovered from urine and faeces within seven days following administration. Eight consecutive daily oral doses of 4.0 mg were quantitatively recovered within 14 days after the last dose (**Neff and Thornton**, 1964a).

Continuous daily oral administration of 4.0 mg of [^{3}H]-melengestrol acetate over 15 days to three Holstein heifers led to a "steady state" and 83 ± 13% of the daily dose was recovered in the urine and faeces on the same day. The animals were killed 1, 4, and 10 days after the last dose and the total residue contents of selected edible tissues were determined (**Neff and Thornton**, 1964b). The results confirmed that the concentrations of total residues in perirenal fat, visceral fat, and omental fat were similar and depleted at similar rates. Even at this eight-fold overdosing no residues above the limit of detection of the radioactive label were found in muscle (see Table 2).

Table 2. Depletion of residues of ^{3}H-labeled melengestrol acetate in selected tissues of heifers.

Withdrawal time (days)	1	4	10
Tissue	Melengestrol acetate-equivalents (µg/kg)		
Liver	43	14	4
Visceral fat	43	22	6
Perirenal fat	43	--	9
Omental fat	42	22	4
Kidney	6	*	*
Heart	2	*	*
loin muscles	*	*	*
Round steak muscles	*	*	*

Note: * = below the limit of quantification (LOQ).

In an attempt to obtain a first estimate of the proportions of the concentrations of the residues in edible tissues, an animal was treated with 1000 mg orally (corresponding to 2000 times the highest recommended dose) for five days. In addition, 500 mg was injected subcutaneously on the fifth day and another 500 mg were injected intramuscularly. The animal was killed on the sixth day and the following concentrations of residues of parent melengestrol acetate were found: fat 3300 µg/kg, liver 880 µg/kg, muscle 220 µg/kg, and kidney 120 µg/kg. These results are in line with the findings that residues in muscle were not detectable in studies when the animals were treated with recommended doses.

TISSUE RESIDUE DEPLETION STUDIES

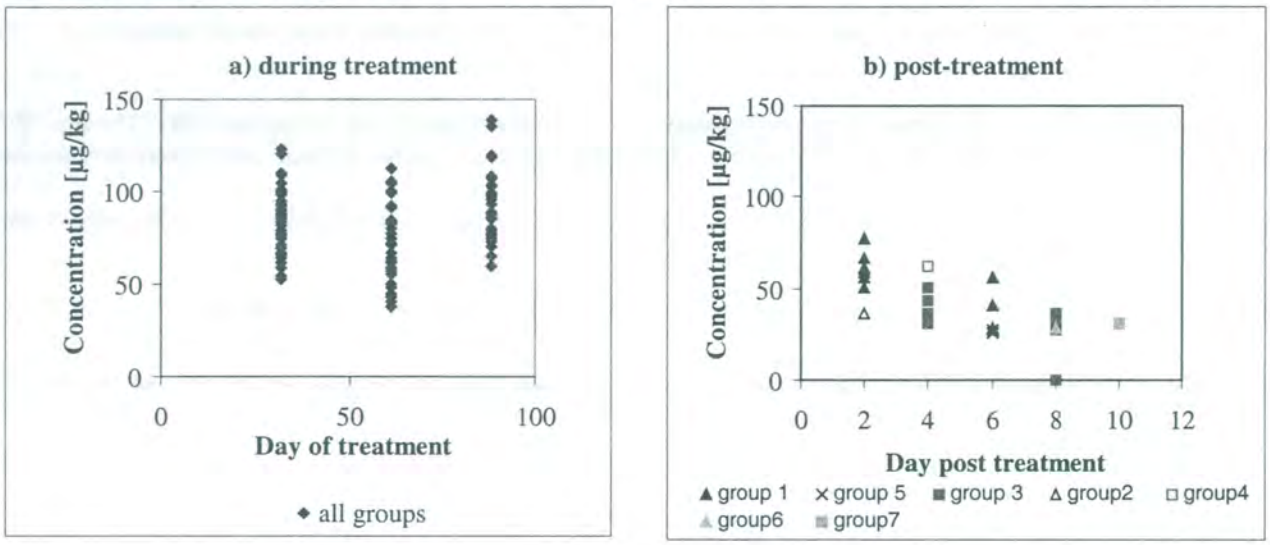
Muscle, liver, kidney and fat obtained from 5 heifers (initial body weights: 234-280 kg; final body weights 320-386 kg) fed 0.5 mg melengestrol acetate/animal daily for 126 days and slaughtered after two days withdrawal contained no quantifiable residues of melengestrol acetate (**Krzeminsky *et al.***, 1971a). The reported LOQ of the method was 25 µg/kg. Similar results were obtained in two similarly designed studies where five beef heifers received 0.5 mg melengestrol acetate/animal daily for 142 and 143 days, respectively, in combination with oxytetracycline and chlorotetracycline, respectively (**Krzeminsky *et al.***, 1971b; **Krzeminsky *et al.***, 1971c). In these studies animals were slaughtered following a two-day withdrawal period. The method used for quantification was GLC with electron capture detection in both studies (**Krzeminsky *et al.***, 1970). The reported LOQ was 25 µg/kg.

In a study with thirty-five Angus beef heifers with an initial average body weight of 241 kg, seven groups each comprising 5 animals were fed 10.0 mg melengestrol acetate/animal daily for 113 days. Biopsies of perirenal fat were taken at 32, 61, 88 days during treatment and 2,4,6,8, and 10 days post-treatment (**Krzeminsky et al.**, 1971d). The results of the quantitative determination of melengestrol acetate residues are given in the Figures 1a and 1b. The concentrations of several samples were below the LOQ of 25 µg/kg (1/10 on day four post-dose; 5/10 on day eight post-dose and 9/10 on day ten post-dose). During treatment the following concentrations were reached (Table 3):

Table 3. Melengestrol acetate in samples of perirenal fat in heifers "on treatment" with 10 mg per day.

Sampling time (days)	arithmetic mean	geometric mean	median
32	85.2	83.3	86.5
61	71.3	68.7	71.0
88	92.6	90.8	95.5
32 to 88	82.9	80.4	82.9
zero withdrawal time (extrapolated)		78.0	

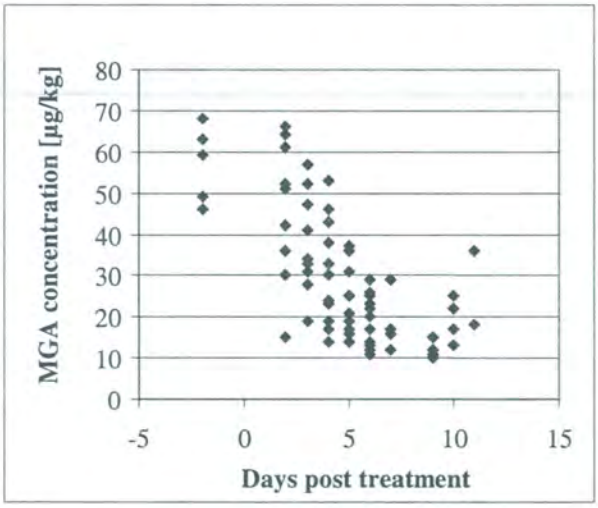
Figure 1a, 1b. MGA residues in peri-renal fat during and post-treatment in cattle receiving 10 mg per day



Eight groups of beef heifers comprising a total of seventy-nine animals were fed 0.4 mg melengestrol acetate/animal daily for 48 days. With forty-seven animals the treatment was continued for 14 days. The 32 remaining animals received reduced doses of 0.25 mg of melengestrol acetate/animal daily during the same period of time. Fat biopsy samples were taken and analyzed at various times (0,1,2,4 and 6 days post treatment in the 0.4 mg dose group and 0,1 and 2 days post treatment in the 0.25 mg dose group). No residues above the LOQ of the method (10 µg/kg) were found in any fat sample (**Krzeminsky *et al.***, 1973a).

One hundred and fifteen beef heifers were fed 3.0 mg melengestrol acetate/animal daily. Biopsies of perirenal fat were taken from six animals after 13 days of treatment. The drug was withdrawn after 15 days of treatment and biopsy samples were taken 2,3,4,5,6,7,8,9,10,11, and 12 days post-treatment (**Krzeminsky *et al.***, 1974b). The samples were analyzed by a GLC/ECD method with an LOQ of 10 µg/kg (**Krzeminsky and Cox**, 1974c). The results are given in Figure 2. The concentrations found during treatment were [µg/kg]: arithmetic mean, 59; median, 61; value at zero withdrawal time (extrapolated by semi-log linear regression from data obtained between days 2 and 6 post-dose), 69.

Figure 2. Residues of MGA in peri-renal fat from animals receiving 3 mg per day



Seventy-one beef heifers were fed 0.4 mg of melengestrol acetate/animal daily. The animals were fed either a high roughage or a high concentrate diet for the first 41 days of treatment. Heifers on a high roughage diet had no residues above LOQ (10 µg/kg) in fat biopsy samples obtained on days 20 and 41 of the treatment. Of the non-pregnant animals fed a high concentrate diet, one of fourteen sampled animals had residues above LOQ on day 20 and one of sixteen animals sampled on day 41 had residues above the LOQ. Of the eleven pregnant heifers in the study (all on high concentrate diet), five had residues above the LOQ in fat biopsy samples on day 41. The study was continued for an additional 83 days with all animals on a high concentrate diet. The animals were then slaughtered at zero withdrawal time or two days after the last dose of melengestrol acetate. Residue concentrations in muscle, liver and kidney were all

below the LOQ. Only one fat sample out of seventy was above the LOQ. The numerical results are summarized in Table 4. Recovery of MGA was approximately 115% at levels between 10 and 30 µg/kg (**Krzeminsky *et al.***, 1973b).

Table 4. Residues of melengestrol acetate in fat biopsy samples of pregnant and non-pregnant animals

Day of treatment	Condition of the animal	MGA [µg/kg]
20	non-pregnant	13.1
41	non-pregnant	10.0
41	Pregnant	11.0
41	Pregnant	20.0
41	Pregnant	11.0
41	Pregnant	13.0
41	Pregnant	10.0
48 hours post-treatment	non-pregnant	11.5

The effects on melengestrol acetate residues in perirenal fat of the melengestrol acetate dose, the type of heifer and the melengestrol acetate withdrawal period was studied. Two doses (0.3 and 0.4 mg/head/day), three withdrawal periods (0, 2, 12 days) were used with light (289-350 kg body weight) and heavy (409-475 kg body weight) commercial feedlot heifers (10 feedlots from 5 States of the United States). Certain test groups received additional growth promotants, e.g., Synovex H/Rumensin, DES-Implant/Rumensin, Synovex H/Ralgro/Rumensin. (**Lauderdale *et al.***, 1977) The range of melengestrol acetate feeding periods is given in Table 5.

Table 5. Range of melengestrol acetate doses and feeding periods in heifers.

Animals	Dose [mg MGA/animal/day]	Feeding period [days]
Light heifers	0.3	131-187
	0.4	100-185
Heavy heifers	0.3	118-147
	0.4	110-180

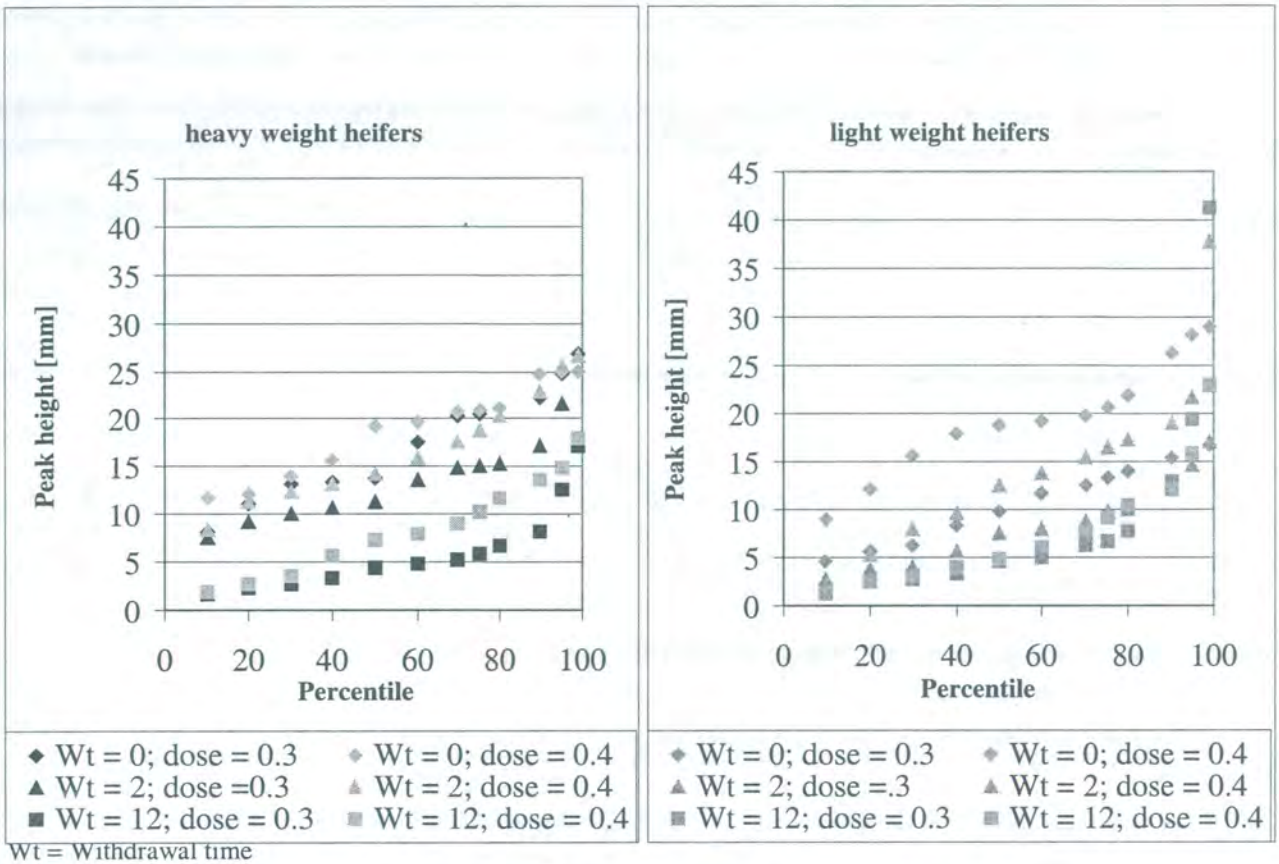
Perirenal fat samples were collected from a total of 481 heifers. The GLC/ECD method with a LOQ of 10 µg/kg was used. All observed peak heights for residues in fat samples were normalized relative to a 10 µg/kg melengestrol acetate standard (20 mm peak height under the specified experimental conditions). These data are summarized in Table 6.

Table 6 Gas-chromatographic peak heights at melengestrol acetate retention time [mm].

	Heavy heifers (409-475 kg bw)						Light heifers (289-350 kg bw)					
Withdrawal time (days)	0		2		12		0		2		12	
Dose (mg/head/day)	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4
Number of animals	12	17	40	60	40	59	18	15	60	50	60	50
Arithmetic mean	15.8	17.7	12.7	15.3	4.90	7.27	9.78	17.9	7.28	12.0	6.62	6.46
Geometric mean	18.8	17.0	11.9	14.4	3.84	5.27	8.73	16.7	6.15	8.90	4.18	4.67
Median	13.8	19.2	11.3	14.3	4.35	7.30	9.75	18.7	7.40	12.6	4.50	4.80
75th percentile	20.5	21.0	15.0	18.8	5.83	10.3	13.3	20.6	9.73	16.5	6.65	9.18
90th percentile	22.2	24.7	17.1	22.8	8.11	13.6	15.4	26.2	12.9	19.0	13.0	12.1
95th percentile	24.6	24.8	21.6	25.6	12.5	14.9	15.6	28.2	14.5	21.7	19.3	15.9
99th percentile	26.8	25.0	26.5	26.6	17.1	18.0	16.6	29.0	17.1	37.7	41.3	22.9

Both parameters, dose of melengestrol acetate and duration of withdrawal time prior to sampling significantly affected the results. The data also suggest a more rapid decline in light heifers if compared with heavy heifers in this experiment (see Figures 3a and 3b).

Figure 3a, 3b. Influence of dose and withdrawal time on MGA residues in heifers



Wt = Withdrawal time

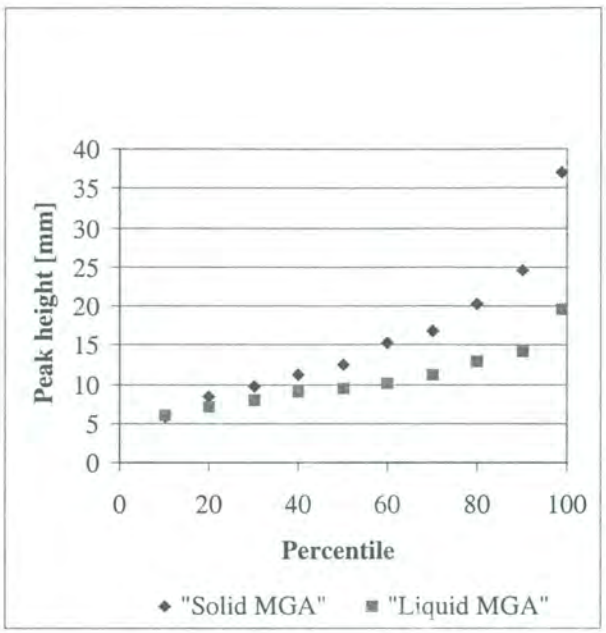
Three groups of heifers (12 animals per group) weighingfrom 300-386 kg were treated with melengestrol acetate, melengestrol acetate plus rumensin, and melengestrol acetate plus Synovex H implants, respectively for 49 days. The melengestrol acetate dose was 0.5 mg/animal daily. Perirenal fat was collected between 49 and 55 hours after complete consumption of the last melengestrol acetate feeding. The 90th percentile of all measured contents was slightly above 10 µg/kg. However, the number of above LOQ results was too small to compare the effects of the different treatments. This was also the case after continuation of treatment for another 36 or 46 days (**Krzeminsky *et al.***, 1977).

Similarly, residues in fat, liver, kidney and muscle were below the LOQ of 10 µg/kg when 0.4 mg melengestrol acetate/head/day was administered in combination with 0.5 ppm of salinomycin, equivalent to 100 mg/head/day (**Davis and Hamlow**, 1992).

Due to a lack of sensitivity of the method applied in some early studies, no results above the LOQ of 25 µg/kg were observed in edible tissues when beef heifers were treated with melengestrol acetate in the presence of zinc bacitracin (**Krzeminsky *et al.***, 1969a). Similarly no residues above the LOQ of 25 µg/kg were found in edible tissues of 201 Hereford yearling heifers which had received 0.5 mg melengestrol acetate/head/day in combination with 350 mg chlorotetracycline/head/day and from which tissues were collected two days post-treatment (**Krzeminsky *et al.***, 1969b). Similarly no residues above the 25 µg/kg LOQ were found in tissue samples of animals treated with a combination of 0.5 mg melengestrol acetate and 75 mg Oxytetracycline per head per day over a period of 100 days and slaughtered after 2, 3, and 4 days withdrawal, resp. (**Krzeminsky *et al.***, 1969c). In another study in which the animals were treated with 0.5 mg melengestrol acetate/head/day in the presence of 350 mg and 70 mg Chlorotetracycline for the first 30 and subsequent 110 days, resp. no tissue residues above the LOQ of 25 µg/kg were found (**Krzeminsky *et al.***, 1969d).

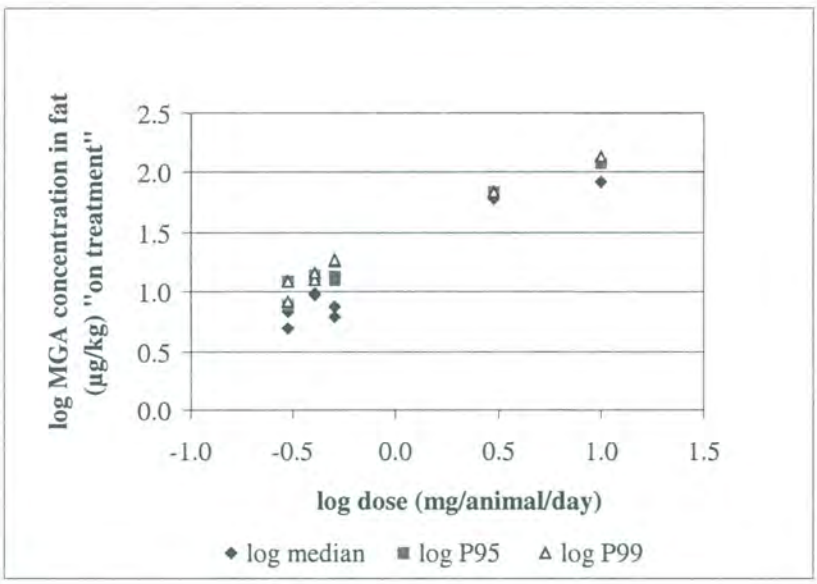
Analyses were carried out on 174 samples of perirenal fat obtained from 25 feedlots where heifers were fed 0.5 mg of melengestrol acetate/animal daily via a conventional supplement. In addition, another 84 fat samples were obtained from 12 feedlots where 0.5 mg of melengestrol acetate/animal daily was fed using a liquid formulation of melengestrol acetate that was delivered into the complete feed by a metering machine (**Krzeminsky *et al.***, 1983). Beef heifers were predominantly a mixed breed. Of the 37 groups of cattle, 27 were slaughtered at less than 10 hrs after the last melengestrol acetate feeding, 8 were slaughtered between 11 and 16 h after the last melengestrol acetate feeding and 2 were slaughtered at 18 and 27.5 h after the last melengestrol acetate feeding. Peak heights were significantly lower (see Figure 4) in samples from animals treated with a liquid formulation poured on feed compared to the administration of the solid melengestrol acetate formulation mixed into feed.

Figure 4. MGA formulation effects on peak height in the GLS/ECD chromatographic analysis of fat samples.



For the population sampled in this study, the 99th percentile of the concentrations of melengestrol acetate was about 18 µg/kg. The median concentration was about 6 µg/kg. A relationship between the administered dose and the concentrations of melengestrol acetate residues found "on treatment" could be established on the basis of the results of the above discussed studies that were conducted over a range of doses from 0.3 to 10.0 mg melengestrol acetate/animal per day (see Figure 5). Using this relationship it was estimated for the lower approved dose of 0.25 mg/head per day, that the corresponding upper 99th percentile would be about 10 µg of parent melengestrol acetate/kg of fat.

Figure 5. Relationship of the administered dose of MGA and concentration of residues in fat.



Marker Residue and Target Tissues

The average theoretical daily intake of "total residues" resulting from the treatment of heifers with 0.5 mg melengestrol acetate/animal per day and slaughtering at zero withdrawal time can be estimated from the data given in Table 1. The results of these calculations are given in Table 7a. It is evident that on the basis of the JECFA food basket liver would be the main dietary source of intake of total residues. A similar calculation based on parent compound melengestrol acetate is carried out in Table 7b. From these tables it becomes evident that liver and fat are equally important as dietary sources of melengestrol acetate parent drug residues. The contribution of muscle and kidney is less significant

compared with liver and fat. The parent melengestrol acetate contents in fat are the highest of all four standard edible tissues. Therefore, parent melengestrol acetate and fat (most data are from perirenal fat) are the suitable pair of marker residue and target tissue, respectively.

Table 7a. Estimation of the dietary total residue intake of melengestrol acetate

Tissue	Content of "total residues" expressed as µg/kg MGA equivalents				Food consumption [g/person/ day]	Intake [µg/person/ day]	% of total daily intake
	animal 1	animal 2	Animal 3	mean			
Fat	7.5	7.7	8	7.7	50	0.39	20.5
Muscle	0.6	1	0.5	0.70	300	0.21	11.1
Liver	12	15.4	9	12.1	100	1.21	64.3
Kidney	1.7	1.8	1.2	1.6	50	0.08	4.1
All tissues						1.9	100.0

Table 7b. Estimation of the dietary parent drug intake of melengestrol acetate.

Tissue	Content of MGA [µg/kg]				Food consumption [g/person/ day]	Intake [µg/person/ day]	% of total daily intake
	animal 1	animal 2	Animal 3	mean			
Fat	5.85	6.62	7.52	6.66	50	0.33	39.5
Muscle	0.19	0.72	0.20	0.37	300	0.11	13.1
Liver	3.60	4.62	2.52	3.58	100	0.36	42.4
Kidney	0.41	0.61	1.51	0.84	50	0.04	5.0
All tissues						0.8	100.0

METHODS OF ANALYSIS

The analytical methods submitted to the Committee were developed before the introduction of GLP. A text published in the US Federal Register (**FDA**, 1968) describes in full detail the original analytical method for determination of residues of melengestrol acetate in lean muscle and fatty tissues. Following the extraction of residues, interfering substances are removed from the extract by several solvent partition and thin layer chromatographic clean-up steps. Final separation and detection is performed by GLC-FID (Flame Ionisation Detector) using a 3% QF-1 phase in an all-glass packed column. Quantification is performed on the basis of peak height measurements . Melengestrol acetate can be quantitatively determined at a level of 25 µg/kg with negligible interference from tissues or reagents. Observed recovery and estimated standard deviation at 25 ppb in muscle , liver and fat is 74.4 ± 8.0 %.

This method has been further developed and GLC-FID detection has been replaced by electron capture detection (GLC-ECD). Furthermore, a GLC-MS method using 25 g of fat and the ions with m/z 311, 321, 336, 337, and 354 amu has been described for confirmation (**Krzeminsky *et al.***, 1974a). A collaborative study of the modified GLC-ECD method with seven participating laboratories (**Krzeminski *et al.***, 1976) gave the results summarized in Table 8.

Table 8. Summary characteristics of the collaboratively studied method for the determination of melengestrol acetate residues in bovine tissues.

Sample		Analytical procedure	Validation parameters		
Matrix	Sample weight [gram]		Concentration [µg/kg]	Limit of quantification [µg/kg]	Recovery at 10 and 20 µg/kg [%]
Kidney	25	Extraction, solvent partition, gas-liquid column chromatography, electron capture detection.	0, 10, 20	10	76-105
Liver	25				78-142
Muscle	25				74-98
Fat	25				88-108

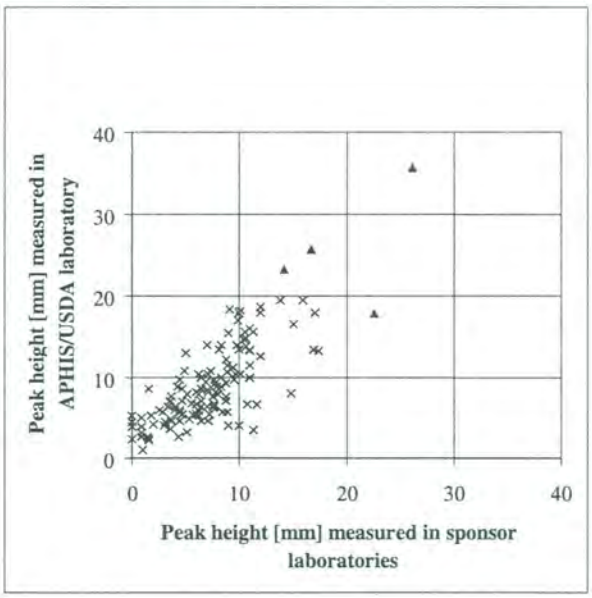
For all four tissues fortified samples were tested at the 0, 10 and 20 µg/kg level; for fat, incurred samples from heifers were tested at the 0, 10 and 20 µg/kg level. Apparent melengestrol acetate concentrations [µg melengestrol acetate equivalents per kg] observed in the zero level control tissues (blanks) ranged between 0.0 – 4.6 (kidney), 0.0 – 4.2 (liver), 0.0 – 1.1 (muscle) and 0.0 – 2.2 (fat). From statistical analyses it was concluded that the true concentration of melengestrol acetate for the incurred fat samples was in the range 6.9 –13.8 µg/kg for the sample at the 10 µg/kg level and in the range 13.4 – 26.4 µg/kg for the sample at the 20 µg/kg level. It was concluded that the method could distinguish samples with residues at the 10 µg/kg level from samples not containing melengestrol acetate residues, but that the method could not fully differentiate between samples with residues at the 10 µg/kg and the 20 µg/kg level. This collaborative study resulted in the AOAC Official Method 976.36 (**AOAC International**, 1995) applicable to animal tissues (kidney, liver, muscle, fat; sample weight 25 gram each) down to 10 µg/kg melengestrol acetate with a calibration range between 10 and 30 µg/kg (first action in 1976 and final action 1978).

The method uses large volumes of organic solvents, amongst others benzene, a solvent the use of which is prohibited in many parts of the world due to its carcinogenic properties. It uses packed column GLC-ECD for final separation and quantification of the melengestrol acetate residue. However, improvements to GLC-ECD instrumentation and its implicit analytical signal processing varied considerable in the past 25 years. The GLC-ECD conditions as used in the collaboratively tested method are no longer operational or even available in contemporary regulatory laboratories performing residue analyses. In consequence the sponsors method as such does no longer exist. The validation data obtained from the 1974 AOAC collaborative study are no longer applicable for contemporary method performance.

As early as the mid seventies various independent attempts were made to develop regulatory methods with better performance characteristics than the sponsors method, especially with regard to improved specificity and a lower limit of quantification (see for example the GLC-ECD method published by **Ryan & Dupont**, 1975). The Committee also noted that a variety of potentially suitable modern regulatory residue methods is available which are *in-house-validated* according to recent requirements (**FAO/IAEA**, 1998).

A collaboratively studied GLC/ECD method (**Krzeminsky *et al.***, 1976) was used in the most relevant residue studies. The results of the GLC/ECD method can be confirmed by a GLC-mass spectrometric method with a LOQ of 10 µg/kg in fat (**Krzeminsky *et al.***, 1974a). Further comparative studies have been conducted in order to correlate the results obtained with field samples collected in several U. S. States and using this method in two or more laboratories, e.g., USDA/APHIS and the sponsor. In one such study (**Neff**, 1977), 130 fat samples obtained from beef heifers were analyzed in the two laboratories. A coefficient of correlation of r=0.791 was obtained. The results are shown in Figure 6 on the basis of normalized peak heights measured (20 mm peak height corresponds to 10 µg/kg melengestrol acetate).

Figure 6. Correlation of the results of MGA assays in fat carried out in two laboratories.



The results of the sponsor laboratories had not been corrected for recovery. The triangles in Figure 6 highlight results that were above the LOQ in at least one of the two laboratories involved in the study. The good correlation between results even at levels below the established lower limit of reliable measurement suggests that melengestrol acetate was present in nearly all fat samples and that the 99th percentile of the residue levels found in these field samples was slightly above 10 µg/kg.

APPRAISAL

A large data base was made available to the Committee to estimate the median and higher percentiles of "on treatment" concentrations of residues of parent melengestrol acetate in fat of animals treated with the upper recommended dose (0.5 mg/animal per day. There were also sufficient data from several studies where doses ranging from 0.3 to 10 mg of melengestrol acetate/animal per day had been used to extrapolate the 99th percentile of "on treatment" concentrations of residues of parent melengestrol acetate in fat of animals treated with the lower recommended dose (0.25 mg of melengestrol acetate/animal per day). There were three studies that included four different dose rates where it was also possible to estimate rate constants for the depletion of parent melengestrol acetate in fat. However, information on concentrations of residues of melengestrol acetate in the remaining three standard edible tissues (muscle, liver and kidney) was limited. Knowledge of the ratio between concentrations of "total residue" and of parent melengestrol acetate was based on a study conducted with four animals. In this study ^{3}H-labeled as well as ^{14}C-labeled melengestrol acetate was used. No information on both the nature and quantities of individual metabolites with potential progestogenic activity was available.

MAXIMUM RESIDUE LIMITS

Therefore, in recommending MRLs, the Committee took into account the following:

- An ADI of 0-0.03 µg/kg of body weight corresponding to an upper limit of acceptable daily intakes of 1.8 µg for a 60 kg body weight person
- Only fat and liver contain routinely quantifiable concentrations of the marker residue; methods with limits of quantification above 0.3 µg/kg are unlikely to quantify residues in muscle of incurred tissues from animals treated with recommended doses of melengestrol acetate. The sponsors did not make available a suitable analytical method for monitoring the MRLs at this concentration.
- Due to lack of information on structure and activity, metabolites had to be treated as if they were equally potent progestogens.

On this basis, the Committee recommended Temporary Maximum Residue Limits of 5 µg/kg of parent melengestrol acetate in fat and of 2 µg/kg of parent melengestrol acetate in liver.

Taking into account the ratio between concentrations of total residue and of marker residue in the four standard edible tissues (see Tables 1, 7a and 7b) a Theoretical Maximum Daily Intake (TMDI) of 1.2 µg of total residue expressed as melengestrol acetate equivalents per 60 kg person would result from the consumption of 300 g of muscle, 100g of liver, 50g of kidney and 50g of fat. The contribution of muscle and kidney to the TMDI is in the order of only 0.15 µg of total residue for muscle and 0.05 µg for kidney. The contribution to this TMDI of the residues of parent melengestrol acetate in all four tissues is about 0.6 µg per person. Therefore, the Theoretical Maximum Daily Intakes of parent melengestrol acetate as well as of its unidentified metabolites remains well below the ADI recommended by the Committee.

REFERENCES

AOAC International (1995). AOAC official method 976.36 Melengestrol acetate residues in animal tissues - gas chromatographic method - first action 1976 & final action 1978. AOAC official methods of analysis, 16th Edition, AOAC International, Arlington VA, USA. Chapter 23: 6 – 8. ISBN 0935584544.

Cooper J.M. (1968). The metabolism of megestrol acetate (17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) and its 16-methylene derivative, melengestrol acetate (17α-acetoxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione). Doctoral thesis submitted to the university of London.

Davis R.A. and Hamlow P.J (1992). The analysis of melengestrol acetate (MGA) in fat, kidney, liver, and muscle tissues taken from feedlot heifers fed MGA and salinomycin in combination.Technical Report No. 623-7926-91-002, 28 February 1992. Agricultural Division, The Upjohn Company Kalamazoo, Michigan.

FAO/IAEA (1998). Validation of analytical methods for food control. Report of a Joint FAO/IAEA Expert Consultation, Vienna, Austria, 2-4 December 1997. FAO Food and Nutrition Paper, Rome 68: pp 19. ISBN 92-5-104131-8.

FDA (1968). US Federal Register February 6, 1968, Part 121-Food Additives, Subpart D. Melengestrol Acetate , paragraph 121.1214 dated January 26,1968; 33: 2602-2605.

Jaglan P.S. (1975a). Metabolism of MGA in beef heifers: V, *in vitro* incubation of MGA with liver homogenates. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan. Report 623-9760-21, 2 July 1975.

Jaglan P.S. (1975b). MGA metabolism study in beef heifers: IV, Metabolism of ^{3}H-MGA in heifer rumen fluid., Agricultural Division, The Upjohn Company, Kalamazoo, Michigan. Report 623-9760-21, 23 May 1975.

Krzeminski L.F., Bloss R.E., Gosline R.E., Subacz C.J., and Thornton A.M. (1969a). MGA residue study in beef heifers receiving MGA-bacitracin . Report No.512-9760-37, 5 March 1969. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminski L.F., Bloss R.E., Gosline R.E., Thornton A.M., and Subacz C.J. (1969b). Tissue residue study in beef heifers treated with MGA-chlorotetracycline. Report 512-9760-41, 18 February 1969. Agricultural Division, The Upjohne Company, Kalamzoo, Michigan.

Krzeminski L.F., Gosline R.E., Thornton A.M., and Subacz C.J. (1969c). MGA residue study in hereford beef heifers receiving MGA-terramycin. Report 512-9760-43, 4 April 1969. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminski L.F., Gosline R.E., Subacz C.J., and Cox B.L. (1969d). MGA residue study in beef heifers receiving MGA-CTC. Report 512-9760-46, 12 November 1969. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan (1969).

Krzeminski L.F., Cox B.L., and Subacz C.J. (1970). Revised short MGA procedure., Report No. 512-9760-51, October 13, 1970.

Krzeminski L.F., Bloss R.E., Cox B.L., Subacz C.J., and Gosline R.E (1971a). Tissue residue study in beef heifers treated with MGA. Project 512-9660-1-REB-70-02. Agricultural Division The Upjohn Company, Kalamazoo, Michigan.

Krzeminski L.F., Bloss R.E., Cox B.L., Subacz C.J., and Gosline R.E (1971b). Tissue residue study in beef heifers fed MGA and oxytetracycline (OTC) in combination. Report No. 512-9760-55). Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminski L.F., Bloss R.E., Cox B.L., Subacz C.J., and Gosline R.E (1971c). Tissue residue study in beef heifers fed MGA and chlorotetracycline (CTC) in combination. Report No. 512-9760-54). Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminski L. F., Bloss R.E., Miller C.C., Subacz C.J., Cox B.L., Bell R.L., and Lallinger A.J. (1971d). MGA fat biopsy study in beef heifers fed 10 mg MGA/head/day. Report No. 512-9760-56. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminsky L.F., Bloss R.E., and Cox B.L. (1973a). MGA fat biopsy study in beef heifers fed MGA. Report 623-9760-2, Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminsky L.F., Bloss R.E., and Cox B.L. (1973b) MGA residue study in beef heifers fed MGA. Project 623-9660-1-REB-72-02. On-treatment and two-day withdrawal study. Report 623-9760-3, Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminski, L.F., Cox, B.L., Boal J.R., and Baczynsyj L. (1974a). Confirmatory GLC-mass spectrometry procedure for MGA in fat at the 10 ppb level. Report No. 623-9760-11. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan, February 26, 1974.

Krzeminski L.F., Cox B.L., and Lauderdale J.W. (1974b). MGA decline study in fat of beef heifers. Report 623-9760-13. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan .

Krzeminski L.F. and Cox B.L. (1974c). MGA assay sensitive to 10 µg/kg. Report 623-9760-9, February 19, 1974.

Krzeminski L.F., Geng S., and Cox B.L. (1976). Determination of melengestrol acetate in bovine tissues: collaborative study. JAOC 59, 507-516.

Krzeminski L.F., Cox B.L., and Lauderdale J.W. (1977). Assay results from the MGA, MGA + rumensin and MGA + Synovex H implant Study. Report No.623-9760-34, 12 July 1977. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Krzeminski L.F., Cox B.L., and Gosline R. E (1981). Fate of radioactive melengestrol acetate in the bovine. J. Agric.Food Chem. 29, 387-391.

Krzeminski L.F., Van Buren J.W., Ash K.A., Davis R.A., Lauderdale J.W., Subacz C.J., and Hoffman G.A. (1983). On treatment levels of MGA® in feedlot heifers in the USA. Technical Report No. 623-9760-83-001. Agricultural Research and Development Laboratories, the Upjohn Company (1983).

Lauderdale J.W., Farho C.J., Krzeminski L.F., and Thomas R.W. (1977). Melengestrol acetate (MGA®) tissue residue field study. Report No. 623-1-9670-77-007. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan.

Neff A.W. and Thornton A.M. (1964a). Oral melengestrol acetate (U-21,240) Tracer Study No.1 in the heifer. Unpublished interoffice memorandum of April 27, 1964. The Upjohn Company, Kalamazoo, Michigan.

Neff A.W., Kleckner M.J., and Thornton A.M. (1964b). Oral melengestrol acetate (U-21,240) Tracer Study No.2 in the heifer. Unpublished interoffice memorandum of November 4, 1964. The Upjohn Company, Kalamazoo, Michigan.

Neff A.W. (1977) Melengestrol acetate (MGA®). Tissue residue surveillance program cooperating with APHIS. Agricultural Division, The Upjohn Company, Kalamazoo, Michigan

Ryan J.J., Dupont J.A. (1975). Measurement and presence of melengestrol acetate (MGA) in beef tissues at low levels. J Agric Food Chem 23; 917-920.

PERMETHRIN

First draft prepared by
Raymond Heitzman, Newbury, United Kingdom
Ludovic Kinabo, Morogoro,Tanzania
Michael Morgan, Leeds, UK

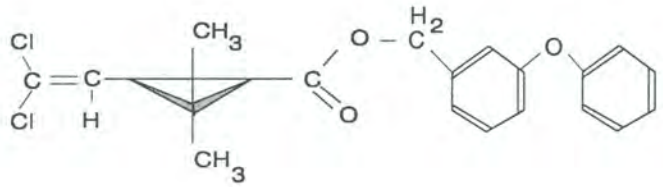
IDENTITY

Chemical Name: 3-phenoxy (±) *cis*, *trans* 3-(2,2-dichlorvinyl)-2,2-dimethylcyclopropane-1-carboxylate (IUPAC).
4-(phenoxyphenyl)-methyl (±) *cis*, *trans* 3-(2,2-dichlorethenyl)-2,2-dimethylcyclopropanecarboxylate (CAS), CAS No. 52645-53-1

Generic Name: Permethrin is an approved name.

Commercial Names: Swift, Rypospect, Flypor, Switch.

Structural formula:



Molecular formula: $C_{21}H_{20}Cl_2O_3$
Molecular weight: 391.3

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Permethrin 80:20 *cis : trans*
Appearance: Pale yellow crystals or yellow to brown oil.
Melting point: 34-39°C for 80:20 *cis:trans* mixture. Pure *cis* 63-65°C; *trans* 44-47°C
Boiling Point: 80:20 *cis:trans* mixture (220°C at 0.5mm Hg)
Solubility: Insoluble in water (0.2 mg/L at 30°C); soluble in hexane, benzene, chloroform, ethanol and acetone.
Partition Coefficient: n-octanol:water ($logP_{ow}$) at 20°C = 6.5
Stability: Moderately stable in the environment with a half lives of 28 days in soil and about 10 days on plants. It is stable in weak acid. Readily hydrolysed in strong acid and base.
Toxicity: The *cis* isomers are approximately 10 times more toxic in acute studies than the *trans* isomers.

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use
The 40:60 *cis:trans* formulation is used mainly as an agricultural pesticide and also as an animal ectoparasiticide. The 80:20 *cis : trans* formulation is used as an ecto-parasiticide pour-on formulation for cattle at a dose rate of approximately 4 mg/kg BW up to a maximum dose of 1.6g per animal.

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics and metabolism of orally administered permethrin in 40:60 *cis:trans* form has been extensively reviewed in the scientific literature and especially by a working party organised by IPCS and jointly sponsored by

UNEP, ILO and WHO (IPCS 1990). The data for farm animals is mostly associated with the administration of the 40:60 *cis*:*trans* form and limited data are available for the 80:20 *cis*:*trans* form.

The important aspect for laboratory animals is the pharmacokinetics and metabolism following oral administration, because this route is to be compared with human consumption. The pharmacokinetics in cattle should also review the fate of the drug when applied externally since this is the route of administration for the ecto-antiparasiticidal action of the drug. However, the target animals are also exposed to this compound in the 40:60 *cis*:*trans* form as a contaminant present on or in plant foods

Laboratory Animals

Rat

Excretion
Preparations of [1RS, *trans*] or [1RS, *cis*] -permethrin (^{14}C-labelled in the acid or alcohol moiety were administered orally to male rats at 1.6 – 4.8 mg/kg BW. The compounds were rapidly metabolised and almost all the radioactivity was eliminated in the urine or faeces within a few days. Within 12 days 79-82 % of the radiolabel residues of the 1RS, *trans*] form were eliminated in the urine and 16-18% in the faeces, whereas from the 1RS *cis* isomer 52-54% of the dose was eliminated in the urine and 45-47% in the faeces. The results are shown in table 1. Residues of the *trans* isomer were more rapidly eliminated than the *cis* isomer.

Metabolism
Analysis of the excreta from the above studies revealed five principle sites of metabolic attack in both permethrin isomers, namely, ester cleavage, oxidation at the *trans* and *cis* methyl of the geminal dimethyl group of the acid moiety, and oxidation at the 2′- and 4′- positions of the phenoxy group. The major metabolite from the acid moiety was chrysanthemic acid (Cl_2CA) conjugated with glucuronic acid and excreted in the urine. This accounted for 50-63% of the dose for the *trans*-permethrin and 15-22% for the *cis* form. Oxidation at either of the gem-dimethyl groups was 4.3-10.4% (*trans*) and 12.2-14.9% (*cis*) and these oxidised products were eliminated in the both urine and faeces as such or as the lactone or glucuronide. The major metabolite from the alcohol moiety was 3-(4′-hydroxyphenoxy)benzoic acid (4′-OH-PBacid) sulfate, accounting for 31-43% of the dose for the *trans* and 20-29% for the *cis*. *Cis*-permethrin also yielded 2′OH-PBacid sulfate (ca. 3%). A major metabolite was Pbacid, either free or conjugated, and accounted for 25-31% of the dose for the *trans* isomer and 6-10% for the *cis* isomer. The metabolites are listed in table 2, comparing the profile in rats with those found in bovine excreta. The residues containing radioactivity were very low in tissues other than fat. In fat there was an accumulation of the *cis* derived compounds (0.46-0.62 mg/kg) but less from the *trans* isomer (Elliott et al., 1976, Gaughan et al., 1977).

Food Producing Animals

Cattle

The studies of Gaughan et al., (1978) examined the pharmacokinetics and metabolism of the *cis*- and *trans* permethrin isomers. Four lactating cows were given an oral dose of about 1 mg/kg BW with three consecutive doses of either ^{14}C-*trans*- or ^{14}C-*cis*-permethrin labelled in either the acid or alcohol moieties. Urine, faeces, milk and plasma samples were collected at regular intervals until the cows were slaughtered at 12 or 13 days after the first dose. Tissue samples were collected for residue analysis. The amount of radioactivity in the plasma reached transient peaks shortly after dosing and then declined to low levels (< 50 µg/L). Higher blood levels were attained with the ^{14}C-*trans*-permethrin labelled in the acid moiety than the other three radiolabelled isomers (Gaughan et al., 1978). More of the *trans* isomer was excreted in the urine than the *cis* isomer. More than half of the dose of both isomers was excreted in the faeces. The results are shown in table 1. The excretion pattern for the *trans* isomer for the cows differed from that of rats and goats in that less of the isomer is excreted in the urine and considerably more is excreted in the faeces. (Gaughan et al., 1978). Almost all the administered radioactivity was recovered in the excreta within 9-10 days after the last dose. Over a 12 day test period only 0.03-0.44% of the oral dose was recovered in the milk for either isomer (Gaughan et al., 1978).

Lactating cows were treated with a pour-on preparation of 80:20 *cis*:*trans* permethrin at a dose of 8 mg/kg BW (twice the recommended dose). Plasma (3 cows) and milk samples from 5 cows were collected at 6 and 24 hours after treatment. No residues were detected (LOD 5 µg/kg) in the plasma or in the milk (Robert Young and Co. Ltd., 1984a).

The absorption of 80:20 *cis*:*trans* permethrin following topical administration to cattle was studied using five bullocks weighing 252–313 kg. They were dosed with 40 mg/kg (approximately ten times the recommended dose) poured along the back-line, and bled at regular intervals up to 168 hours after dosing. No residues of parent drug were detected (LOD 5 µg/kg) in any plasma sample (Robert Young and Co. Ltd., 1985).

Metabolism
The metabolism of permethrin in cows follows the same principle routes as that found in the rat and goat. In comparison with rats (Elliott et al., 1976, Gaughan et al., 1977), cows excrete a larger proportion of ester metabolites including the glucuronides (unique in utilising glutamic acid for conjugation) of the carboxylic acid metabolites, and more extensive hydroxylation of the *trans* methyl group and less on the phenoxy moiety. The profile of metabolites in excreta, measured as recovered radioactivity, was: permethrin 5.6% (*trans*), 9.3% (*cis*), hydroxy-permethrin metabolites, 38-40%, metabolites of the acid moiety 54-56% (of which Cl_2CA-glucuronide was 19.2% for *trans* isomer and 4.1% for the *cis*) and metabolites of the alcohol moiety were 55-61% (Gaughan et al., 1978). One of the effects of these differences is that 4′-OH-PB-acid-sulfate is a major metabolite in rats but not in cows.

The calculation of the ratio of unmetabolised permethrin to the total radioactivity residues was not possible for the edible tissues. However, permethrin residues were present in liver, fat and milk.

Goat

Excretion
Goats were treated at a dose of 0.2-0.3 mg/kg BW on ten consecutive days with ^{14}C-*trans*- and ^{14}C-*cis*-permethrin labelled in either the acid and alcohol moiety. The goats excreted most of the *trans* isomer in the urine and at least half of the *cis* isomer in the faeces (Hunt & Gilbert, 1977). The results are shown in table 1.

Milk
The amount of radiolabel appearing in the milk was <0.7% of the administered dose. A larger amount of the *cis* isomer was present as parent compound than for the *trans* isomer.

Tissues
Most of the radioactivity in the fat tissues was parent compound(s) or ester metabolites (e.g., *trans*-OH-permethrin or the glucuronide) (Ivie & Hunt, 1980).

Metabolism
The principle metabolites found in the excreta were the same as those found in bovine excreta (Ivie & Hunt, 1980).

Table 1. Excretion of radioactivity as a percentage of dose in animals after oral dosing of either *cis*- or *trans*- ^{14}C-permethrin.

Species	*Cis* Isomer		*Trans* Isomer	
	Urine	Faeces	Urine	Faeces
Rat	52-54	45-47	79-82	16-18
Cow	22-28	60-76	39-47	52-57
Goat	25-36	52-68	72-79	12-15

Table 2. Metabolites as a percentage of dose in the excreta of rats and cows after oral dosing with either *cis*- or *trans*- ^{14}C-permethrin labelled in the acid or alcohol moieties.

Metabolite	*Cis*-isomer		*Trans*-isomer	
	Rat	Cow	Rat	Cow
Permethrin	12	9	5-7	6
OH-permethrins	17-19	29	0	34
$CACl_2$ (+conjugates)	15-22	5	50-63	21
OH- $CACl_2$	12-15	13	4-10	15
PB-alcohol (+ glucuronide)	20-29	9	31-43	11
PB-acid metabolites	6-10	17	25-31	41

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

The radiolabelled studies in cattle to date have been associated with the oral administration of ^{14}C-labelled permethrin and no studies are yet available for the 80:20 *cis:trans* permethrin. Four lactating cows were treated at a dose of about 1 mg/kg BW with three consecutive doses of either ^{14}C-*trans*- or ^{14}C-*cis*-permethrin labelled in either the acid or alcohol moieties. Urine, faeces, milk and plasma samples were collected at regular intervals until the cows were slaughtered at 12 or 13 days after the first dose. Tissue samples were collected for residue examination (Gaughan et al., 1978). The total residues in muscle, kidney, brain, heart and skin were all below 56 µg/kg, the LOQ for the analytical method. Residues were present in fat and liver and are shown in table 3. The residues of the *cis* isomer were higher than those for the *trans* isomer.

The total residues in milk reached maximum levels around the time of the third dose (day3). The approximate maximum levels were 260 µg/kg and 150 µg/kg for the *trans* and *cis* isomers labelled in the alcohol moiety, respectively, and 20 µg/kg and 200 µg/kg for the *trans* and *cis* isomer labelled in the alcohol moiety, respectively. The levels declined to <10µg/kg by day 12-13 except for the *trans* isomer labelled in the alcohol moiety which declined to about 50 µg/kg by day 10, the last day measurements were made for this isomer (Gaughan et al., 1978). The authors concluded that most of the residues were likely to be unmetabolised permethrin.

Table 3. Residues of ^{14}C-permethrin in µg/kg equivalents in cows after oral dosing.

Tissue	*Trans*-isomer		*Cis*-isomer	
	^{14}C in acid	^{14}C in alcohol	^{14}C in acid	^{14}C in alcohol
Kidney Fat	<35[1]	109[1]	335[1]	119[1]
Subcutaneous. Fat		<56		101
Visceral Fat	<35[1]	96[1]	202[1]	95
Liver	72 (19)	122 (83)	210 (55)	158 (81)

Footnote 1. Mostly or entirely unmetabolised parent drug.
The value in parentheses is the amount of extractable permethrin residues in µg/kg equivalents.

Radiodepletion studies are ongoing for the topical administration of an 80:20 *cis:trans* permethrin mixture as a pour-on preparation for cattle (sponsor, private communication).

Residue Depletion Studies with Unlabelled Drug

Three early studies and two recent studies are reported by the sponsor in which a pour-on preparation using 80:20 *cis:trans*-permethrin was applied to female cattle (Robert Young and Co Ltd. 1984a, 1984b, 1985, Borthwick, 1995 and Gibson, 1996). The residues of 40:60 *cis:trans*-permethrin following topical and parental administration were reviewed by JMPR (1987) and IPCS (1990).

Residues of 80:20 *cis:trans*-permethrin in Cattle

Cattle were treated with a pour-on preparation of 80:20 *cis:trans* permethrin at a dose of 8 mg/kg BW (twice the recommended dose). Plasma and milk samples were collected at 6 and 24 hours after treatment. Animals were slaughtered in treatment groups of three at 3 days and 7 days post dosing. No residues were detected (LOD 5 µg/kg) in the plasma or milk samples. No residues were detected in muscle, liver and kidney (LOD 5µg/kg) or in fat (LOD 10 µg/kg) (Robert Young and Co. Ltd., 1984a).

Thirty mixed breed female cattle about one year of age were dosed with a pour-on preparation of 80:20 *cis:trans* permethrin at a dose of 6 mg/kg BW (1.5 times the recommended dose). Groups of five heifers were slaughtered at 1, 7, 28, 42, 56 and 77 days after dosing. Tissue samples were collected and analysed for residues of permethrin by a gas chromatography technique. The residues in fat are shown in table 4. The residues persisted in all three fat types, subcutaneous (s.c.), renal and omental fat, but were not detectable in any kidney sample at any time nor in muscle and liver samples by day 28. Whereas there were no residues in the fat at 7 days post dosing in the 1984 study (see above) the residues were at their maximum levels in this study at 7 days; they were 100 ± 39, 157 ± 48 and 137 ± 41 µg/kg in s.c., omental and renal fat, respectively. Residues were only >LOQ (25 µg/kg) in one muscle sample (52 µg/kg) and in one liver sample (31 µg/kg) at day 7 in the same cow (Gibson,1996).

Table 4. **Residues in the fat of heifers after treatment with a pour-on preparation of 80:20 *cis:trans* permethrin at a dose of 6 mg/kg BW.**

Days after dosing	Subcutaneous fat (μg/kg)	Omental fat (μg/kg)	Renal fat (μg/kg)
1	<13, <13, <25, <13, <13	25, <25, <25, 49, <25	<25, <25, 43, <25, <25
7	120, 63, 53, 131, 135	172, 226, 136, 154, 96	144, 193, 147, 118, 81
28	31, 50, 25, 55, 80	121, 78, 149, 118, 86	146, 86, 227, 149, 120
42	<13, <25, <13, <25, 130	121, 127, 132, 129, 192	175, 211, 169,127, 241
56	41, <13, <13, <25, 95	117, 65, <13, 107, 177	110, 83, 39, 155, 216
77	<13, <13, <13, <13, <13	30, 62, 44, 36, 87	32, 44, 44, 31, 72

Note. <13 is <LOD; <25 is <LOQ.

Eight dairy cows, 500-695 kg, were treated with a pour-on preparation of 80:20 *cis:trans* permethrin at a dose of 1.6 g (2.3-3.2 mg/kg BW). Milk samples were collected twice daily after treatment up to 106 hours after dosing. A mid-sample was collected on the day of treatment (Borthwick, 1995). The individual milk samples and pooled milk samples at a given time after dosing were analysed for residues of permethrin by a gas chromatography method. The results are shown in table 5. The dose used is that recommended by the sponsor but because of the high weight of the dairy cows the dose is much lower than 4 mg/kg B.W. recommended for lighter animals.

Table 5. **Residues in milk samples (μg/kg) after treatment of dairy cows with 1.6 g of a pour-on preparation of 80:20 *cis:trans* permethrin.**

Cow	Hours after Dosing											
	0	1	6	10	25	34	49	58	73	82	97	106
1	<2.5	<2.5	<2.5	6.1	7.1	11.3	<5.0	9.5	10.0	5.2	<5.0	<2.5
2	<2.5	<2.5	<2.5	<2.5	<2.5	<5.0	<2.5	5.4	<5.0	<5.0	<2.5	6.7
3	<2.5	<2.5	<2.5	<2.5	<2.5	<5.0	<2.5	<5.0	<2.5	<2.5	<2.5	<2.5
4	<2.5	<2.5	<2.5	<5.0	<5.0	<5.0	<2.5	<5.0	<5.0	<5.0	<2.5	<2.5
5	<2.5	<2.5	<2.5	6.7	8.4	10.6	6.5	11.8	11.2	8.1	6.3	<2.5
6	<2.5	<2.5	<2.5	<2.5	<2.5	<5.0	<2.5	<5.0	<2.5	<2.5	<2.5	10.0
7	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
8	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Pooled	<5.0	<5.0	<5.0	<5.0	3.5	6.7	4.4	6.2	2.6	5.0	<5.0	2.9

Note. The LOQ = 5μg/kg; the LOD = 2.5 μg/kg

Residues of 40:60 *cis:trans*-permethrin in Cattle

Oral Administration. Groups of three cows were dosed orally with 40:60 *cis:trans* permethrin at rates of 0.2, 1, 10, 50 or 150 mg/kg BW for 28-31 days. Mean plateau levels in whole milk were <10 μg/kg at the lowest dose and 300 μg/kg at the highest dose. The residues in milk from the highest dose treatment declined to <10 μg/kg 5 days after dosing. The cattle were sacrificed at the end of the dosing period and residues of permethrin in perirenal fat were <10-40 μg/kg and 2800-6200 μg/kg for the lowest and highest doses, respectively (Edwards and Iswaran, 1977).

Topical Administration. Cows were given six whole body sprays of 1g permethrin per cow at 14 day intervals and also exposed to a self-oiler containing a solution of 0.3 g permethrin/litre. The cows were housed in premises that were sprayed six times at 0.06 g per square meter at 14 day intervals. This treatment is almost certainly at the high end of normal husbandry practice. The cows were sacrificed 5 days after the last topical dose. Residues in muscle, liver and kidney were <10 μg/kg, 100 μg/kg in intestinal fat and 40 μg/kg in subcutaneous fat (Ussary and Braithwaite, 1980).

BOUND RESIDUES AND THEIR BIOAVAILABILITY

The extractable and non-extractable residue portions of ^{14}C-permethrin in edible tissues were measured only in bovine liver by Gaughan et al. (1978). Using the results for the extractable fractions shown in table 3 the non-extractable fractions in liver at 12-13 days after initial oral administration of ^{14}C-permethrin are shown in table 6. The results were similar for the *cis* and *trans* isomers although the nature of the non-extractable residues was not investigated. There was accumulation of non-extractable residues in the liver particularly if the label was in the acid moiety. The rapid metabolism of permethrin in the liver may be followed by either the smaller molecules being incorporated into other molecular components of the liver or a very strong binding of the metabolites to the cellular components. There is no information on the bound residues of the 80:20 *cis:trans* permethrin preparation.

Table 6. The non-extractable residues in bovine liver after oral administration of ^{14}C-permethrin isomers labelled in the acid or alcohol moiety.

Metabolite	Total Residues (µg/kg)	Extractable (µg/kg)	Non-extractable (µg/kg)	% Non-extractable
Trans acid ^{14}C	72	19	53	74%
Cis acid ^{14}C	210	55	155	74%
Trans alcohol ^{14}C	122	83	39	32%
Cis alcohol ^{14}C	158	81	77	49%

METHODS OF ANALYSIS

Two reports have been submitted by the sponsors; Hanif, Z.P. (1995a and1995b). In addition an Expert Report, produced as part of a Codex submission, includes comments on the analytical methodology.

Bovine Tissue

Method Summary. Samples were extracted in boiling hexane/acetone and subjected to various solvent partitions before further clean-up on a florisil column and gas chromatographic analysis with an electron capture detector.

QA System. No mention was made of GLP, NAMAS, or any other system in the documentation.

Matrices. The assay was applied to bovine muscle, liver, kidney and fat.

Accuracy/Recovery. Three fortification concentrations were used for recovery determinations, 25.4, 50.8 and 101.6 µg/kg. For muscle the recoveries and coefficient of variation (CV), at each respective level, were 85.5% (17.0%), 85.4% (13.2%) and 85.7% (14.1%). Similarly, for liver, the recoveries were 109.2% (13.9%), 105.2% (9.1%) and 106.9% (10.6%). For kidney tissues, the recoveries were 105.0% (10.2%), 91.6% (7.4%) and 88.4% (13.9%). For fat, the recoveries were 76.2% (13.9%), 103% (12.4%) and 78.1% (18.6%). (n=6, at each fortification level).

Linearity. The linearity of response of the detector was assessed with standard solutions over the range of 2-500µg/l. The units of the graph of the regression analysis are not clear and, therefore, it is difficult to establish linearity at the lower range.

Sensitivity. Fortifying blank tissue with 12.7 µg/kg of permethrin gave recoveries of 100.7% (muscle; n=3), 115.9% (liver; n=3), 101.6% (kidney; n=3) and 112.7% (fat; n=3). The sensitivity was defined as 12.7 µg/kg.

Specificity. Blank tissue samples were extracted and shown to be free of interfering peaks.

Conclusion. The sponsor method has not been validated to modern standards according to the data provided. Similar analytical methodology for determination of permethrin in bovine tissues reported in the literature has been collaboratively tested.

Bovine Milk

Method Summary. Milk samples were extracted with hexane, cleaned-up with solvent elution from a florisil cartridge and subjected to gas chromatographic analysis using an electron capture detector.

QA System. No mention was made of GLP, NAMAS, or any other system in the documentation.

Accuracy/Recovery. Four fortification concentrations were used for recovery determinations, 4.95, 9.9, 19.8 and 49.5 µg/l. Recoveries and coefficient of variation (CV) were, respectively (lowest level first), 85.1% (7.9%), 73.2% (14.6%), 82.1% (15.8%) and 88.0% (12.6%). (n=6 at each level).

Linearity. The linearity of detector response was examined with standards over the range of 2-500 µg/kg.

Sensitivity . Fortifying blank milk with 2.48 µg/l permethrin gave a mean recovery of 97.5% (n=3). The sensitivity was defined as 2.48 µg/kg

Specificity. Blank milk samples were extracted and shown to be free of interfering peaks.

Conclusion. The method has not been validated to modern standards according to the data provided.

APPRAISAL

Important data are missing. In particular, the toxicity data on a different isomer ratio and a radiodepletion study using the proposed formulation(s). The majority of the older data has been submitted for the 40:60 *cis:trans* permethrin preparation. This was extensively reviewed by JMPR and the IPCS. An ADI of 0-300 µg/60 kg person was established in 1987. MRLs were recommended for a variety of plant foods but not for meat and milk because of insufficient data. However, the sponsor is submitting data for the 80:20 *cis:trans* permethrin preparation that it has developed and is a more toxic and more active mixture than the 40:60 *cis:trans* permethrin. The *cis* form is much more active and more toxic than the *trans* and thus ADIs and MRLs may be different for the two preparations.

The residue depletion data using radiolabelled permethrin is weak for both preparations. In the case of 40:60 *cis:trans* permethrin much of the information is obtained following oral treatment with only one study where the animals and their premises were treated with a large excess of the recommended dose. There is virtually no information on the nature of the total residues in edible tissues or milk for either preparation, except they do appear to be at low levels. This information would assist in choosing a Marker Residue. The Committee was made aware of an ongoing radiodepletion study for the 80:20 *cis:trans* permethrin preparation.

The studies with unlabelled permethrin using a pour-on preparation indicate that residues as the sum of the *cis* and *trans* parent isomers are almost not detectable in muscle, liver and kidney at doses 1.5-2 times higher than the recommended dose. In the most recent residue study the residues persisted in all three fat types, subcutaneous (s.c.), renal and omental fat. This is in contrast with the 1984 study where no residues were detected in the fat at 7 days post dosing, while the residues were at their maximum levels in this 1996 study (100 ± 39, 157 ± 48 and 137 ± 41 µg/kg in s.c., omental and renal fat, respectively). Residues were only above the LOQ (25 µg/kg) in one muscle sample (52 µg/kg), and in one liver sample (31 µg/kg) at day 7 in the same cow (Gibson, 1996). Residues were not detectable in any kidney sample at any time nor in muscle and liver samples by day 28. It is not clear why there are residues in the fat of one study and not the other. The older studies did not find residues in the milk after topical administration but the new study found low concentration of permethrin residues in the milk of 4 of the 8 cows treated with the pour-on preparation. The Committee should consider the two new GLP compliant studies the more reliable.

If an ADI is established and the majority of the metabolites can be shown to have very low toxicity then practical MRL for the parent drug could be recommended in fat. Otherwise if the metabolites are toxic or their toxicity is unknown no MRL can be recommended until the ratios of MR to TR are established in the edible tissues of cattle. No information is yet available on the nature of the bound residues.

The analytical methodology is satisfactory for measuring the residues of parent drug.

MAXIMUM RESIDUE LEVELS

JMPR (1987) established an ADI in 1985 of 0-50 µg/kg BW for the 40:60 *cis:trans* permethrin formulation. The Committee concluded that the available JMPR database was not adequate to assess the toxicity of the 80:20 mixture proposed for use as a veterinary drug. In the absence of an ADI, the Committee was unable to recommend MRLs for the 80:20 *cis:trans* mixture of permethrin.

REFERENCES

Borthwick, H.S. (1995). 4% high *cis* permethrin pour-on; milk residue study in cattle. Grampian Pharmaceuticals Report No. GP95026.

Edwards, M.J. and Iswaran, T.J. (1977). Permethrin: Residue transfer and toxicology study with cows fed treated grass nuts. (Report No. TMJ1519/B) (unpublished report submitted to WHO by ICI Plant Protection Division).

Gaughan, L.C., Ackerman, M.E., Unai, T., Casida, J.E., (1978). Distribution and metabolism of *trans*- and *cis*-permethrin in lactating Jersey cows. J. Agric Food Chem., 26, 613-618

Gibson, N.R. (1996). 4% high *cis* permethrin pour-on; tissue residue study in cattle. Grampian Pharmaceuticals Report No. GP96017.

Hanif, Z.P. (1995a). Analysis of permethrin in bovine milk by capillary gas chromatography (Grampian Pharmaceuticals Method Reference MPE/95/24)

Hanif, Z.P. (1995b). Analysis of permethrin in bovine tissue by capillary gas chromatography (Grampian Pharmaceuticals Method Reference MPE/95/32).

Hunt, L.M. and Gilbert, B.N. (1977). Distribution and excretion rates of ^{14}C-labelled permethrin isomers administered orally to four lactating goats for 10 days. J.Agric. Food. Chem., 25, 673-676.

IPCS (1990). Environmental health criteria for permethrin. No. 94 WHO. 1-125

Ivie, G.W. and Hunt, L.M. (1980). Metabolites of *cis*- and *trans*-permethrin in lactating goats. J.Agric. Food. Chem., 28, 1131-1138.

JMPR (1987). JMPR Pesticide residues in food. FAO Plant Production and Protection Paper. No. 84.

JMPR (1979). JMPR monograph for permethrin 369-425, submitted by sponsor.

Robert Young and Co Ltd. (1984a). Residues in cattle tissues following topical treatment with 4% *cis,trans*-permethrin (80:20). Final report DH/84/35. Vol 2, pp37-43

Robert Young and Co Ltd. (1984b). Residues in cattle milk following topical treatment with 4% *Cis,trans*-permethrin (80:20). Final report DH/84/30. Vol 2, pp35-36

Robert Young and Co Ltd. (1985). Pharmacokinetics of high *cis* permethrin in cattle. Final report MPT/85/1. Vol 2, pp20-36

Ussary, J.P. and Braithwaite, G.B. (1980). Residues of permethrin and 3-phenoxy-benzyl alcohol in cow tissues. (Trial No. 35NC79-001. Report No.TMU0493B submiited by ICI Americas Inc. to WHO).

PERMETHRIN

First draft prepared by
Denis Hamilton, Queensland, Australia
Susan Calumpang, Manila, Philippines

Permethrin, a pyrethroid insecticide, has been evaluated at each JMPR Meeting from 1979 to 1989 and 1991. Technical grade permethrin contains four stereoisomers deriving from chirality of the cyclopropane ring at the C-1 and C-3 positions. Glenn and Sharpf (1977) have shown that the ratio of *cis* to *trans* isomers varies with the method of synthesis. It is desirable to produce different *cis/trans* ratios for certain insecticidal applications (e.g., lower *cis/trans* ratios for animal health products). It is therefore important to note the isomer ratios in products used in the supervised trials and metabolism studies. *Cis*-permethrin is more insecticidally potent than the *trans* isomer. The isomers also differ significantly in rates of photolysis and hydrolysis, in biotransformations and in bioaccumulation. The conclusions and recommendations of the 1979 JMPR meeting are based entirely on agricultural and horticultural uses of technical grade permethrin containing *cis/trans* isomers in approximately a 40:60 ratio. Furthermore, the term permethrin from the 1979 and 1980 JMPR reports relates only to this mixture.

The four major manufacturers of permethrin jointly submitted information to the 1979 meeting (Manufacturers, 1979) indicating that the technical grade products of any of the four manufacturers also meet the following general specifications:

1. Purity not less that 89% permethrin (typically 91-93%); (ii) Physical state: yellow-brown to brown oily liquid; (iii) specific gravity =1.214; (iv) Solubility: easily soluble in hexane, benzene, chloroform, ethanol and acetone. Solubility in water <1 mg/kg, and each impurity present at <2%.

USE PATTERN

Permethrin is effective at low rates against a wide range of *Lepidoptera*, *Hemiptera*, *Diptera* and *Coleoptera*. Unlike natural pyrethrins and earlier synthetic pyrethroids, permethrin is photostable that allows it to be used effectively in agriculture. It has adulticidal, ovicidal and particularly larvicidal activity and is effective against the great majority of insects that have become resistant to standard treatments such as organochlorines and organophosphates (JMPR, 1980). Permethrin is not plant systemic and has very little fumigant and translaminar activity; a program of sprays is usually required (JMPR, 1980).

In reviewing pre-harvest uses, including those on forage crops, the 1979 JMPR noted that in several countries target pests and use patterns are well defined, and pre-harvest withholding intervals reflecting good agricultural practices can be specified. However in many countries where climatic conditions are conducive to a rapid build-up of insect infestations, flexibility in treatments may be necessary for full effectiveness. Therefore, in many countries no pre-harvest withholding intervals are specified. This is made possible by a combination of the low effective use rates and resulting comparatively low residues immediately after spraying. Data indicate that permethrin residues on sprayed crop plants decline relatively slowly. Therefore, any benefit of a pre-harvest withholding interval as a means of reducing residue levels tend to be less than what accrues (JMPR, 1980).

In East Africa, permethrin (3 g ai/t) is used in combination with pirimiphos-methyl (16 g ai/t) as a grain protectant. In French-speaking Africa the treatment rates for bulk maize are 1.5 g ai/t and 8 g ai/t for permethrin and pirimiphos-methyl respectively. In Spain, permethrin is used as a grain protectant at 0.9-1.2 g ai/t with pirimiphos-methyl at 3-4 g ai/t. Permethrin is used as a grain protectant in Australia in combination with piperonyl butoxide. It is recommended for use at 1 and 0.5 g ai/t for 3-9 months and less than 3 months storage respectively (JMPR, 1991).

Permethrin can also be used as a dust, spray or dip to control various ectoparasites of cattle, horses, sheep, pigs and poultry. It can be applied either directly to animal, to buildings in which they are housed or to insect breeding and resting sites (JMPR, 1980).

RESIDUES FROM SUPERVISED TRIALS

Pre-harvest

A large body of residue data from supervised trials was reviewed which noted that permethrin and its metabolites are effectively non-systemic in plants. Residues are highest when crop parts are exposed to the spray as in the case of

forage crops. Residue levels decline comparatively slowly - the half-lives vary from about 1-3 three weeks depending on the crop. The major degradation products are the *cis* and *trans*-isomers of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVA) plus 3-phenoxybenzyl alcohol (3-PBAlc), present primarily as conjugates (and also identified as animal metabolites). In forage crops such as alfalfa, residue levels of the metabolite DCVA and 3-phenoxybenzyl alcohol are small compared with the corresponding permethrin residues. This pattern was found on a range of crops reviewed by the 1979 JMPR. In addition to crops grown specifically for forage, livestock feed can also contain by-products of food processing such as apple pomace and cotton cake) (JMPR 1980).

Residues

Cotton, oilseeds and other field crops

In cotton where levels in the seeds are influenced by the degree of protection by the boll during late season spraying, residues were generally below 0.1 mg/kg. Samples analyzed were the ginned (undelinted) seed. The highest value reported at effective use rates is 0.27 mg/kg. At effective use rates, maximum residues reported were 0.05 mg/kg in soybeans, 0.07 mg/kg in sweet corn kernels, 0.08 mg/kg in peas and less than 0.01 mg/kg in peeled coffee beans. Sprays are normally applied to oil seed rape seven weeks or more before harvest. Residues in the oil seeds were non-detectable (less than 0.01 mg/kg).

Legume vegetables

Residues in Phaseolus beans, which are generally eaten in the pod, are higher than those in soybeans or peas where the seeds are protected from the spray. Mean residues of 0.1-0.2 mg/kg in Phaseolus compare with less than 0.1 mg/kg in soybeans and in peas.

Pome fruits, stone fruits, citrus, berries and other fruits

Considerable residue data are available on apples, on which the rate of residue decline tends to be smaller than on varieties of vegetables. At effective use rates, residues were below 2 mg/kg. Similar patterns were seen on pears, peaches and cherries, although levels on plums were 0.1 mg/kg or less. In oranges, melons and kiwifruits, residues were found almost exclusively in the peel; in edible flesh levels were not found to exceed 0.03 mg/kg. As the data for citrus were confined to a single study with oranges in Spain, the results from supervised trials with other citrus fruits in other countries were considered to be desirable.

Post-harvest uses

Grains

The only significant post-harvest use of permethrin is its application to bulk stored grain. This has undergone extensive laboratory studies and silo-scale trials for this purpose in Australia. All the residue studies show that permethrin is persistent on grains under the prevailing conditions of temperature and moisture content in Australian storage. Initial residues on grain were about 20% below the level expected from the amount applied. Residues decline very slowly in storage; about 80% of the initial (1 month) residue in grain remains after 6-9 months. This level of persistence is found consistently in studies on wheat, barley and sorghum, and probably could be generalized for all stored grain. Studies show also that the initial ratio of *cis/trans* isomers is not changed during 8 months of storage.

Animals

Cows

Cows were given five whole-body sprays of permethrin at a rate of 1.0 g ai/cow, with 14 days between sprays, using a 5% emulsifiable concentrate formulation. Cows were allowed free access to a self-oiler containing a 0.03 g ai/l solution, ensuring at least two applications per day for a period of ten weeks. Cows were housed in premises that were sprayed at a rate of 0.06 g ai/m^2, five sprays taking place with a 14-day interval between sprays; cows had free access to the premises during spraying. This exposure level is at the high end of the range that is likely to occur in normal husbandry practice. Milk samples were taken for analysis before and during the ten days after the fifth application. Only four of 70 milk samples had measurable residues of permethrin or its metabolites *cis*, *trans*-3-(2,2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylic acid (DCVA), 3-phenoxybenzoic acid (3-PBAcid) and 3-phenoxybenzylalcohol (3-PBAlc) (Ussary and Braithwaite, 1980a).

Cows were given 6 whole-body sprays of permethrin at a rate of 1.0 g ai/cow with 14 days between sprays. They were allowed free access to a self-oiler containing a 0.03 g ai/l solution ensuring at least two applications per day for a period of ten weeks. The cows, housed in premises that were sprayed at a rate of 0.06 g ai/m^2 with six sprays taking place with

a 14-day interval between sprays, had free access to the premises during spraying. This exposure level is at the high end of what is likely to occur in normal husbandry practice. Cows were slaughtered five days after the sixth application. Permethrin levels in muscle, liver the kidney were low (<0.01 mg/kg). The highest levels of permethrin were 0.10 mg/kg and 0.04 mg/kg in the intestinal and subcutaneous fat, respectively (Ussary and Braithwaite, 1980b).

Pig

Pigs were housed in premises treated with mist applications of 0.06 g permethrin per m^3 at 14-day intervals and slaughtered one day after the sixth application. Permethrin residues were not measurable in skin, liver or kidney tissues (limit of determination: 0.01 mg/kg) and only low levels of permethrin were found in the fat and muscle tissues (0.02 and 0.01 mg/kg, respectively) (Ussary and Braithwaite, 1980c).

Chicken

Hens were present at the times of application when premises received six mist applications of 0.06 g permethrin per m^3 at 14-day intervals. Eggs were collected at intervals up to 50 days following the first application and hens were sacrificed five days after the sixth application. Eggs were found to contain permethrin at 0.02 mg/kg on one occasion only and below 0.01 mg/kg in muscle, skin, liver and eggs at all other times. A residue of 0.02 mg/kg was detected in fat tissues (Ussary and Braithwaite, 1980d).

Processing studies

The fate of permethrin residues during the processing of cotton, soybeans, apples, pears, grapes and tomatoes was reviewed at the 1979 Meeting. Residues in the seeds of cotton and beans of soya, and in their respective processing fractions used in animal feeds, are well below 0.5 mg/kg (FAO/WHO, 1980).

Apples, Pears and Grapes

Residues in dried apple pomace were 25-30 times the levels in corresponding whole apples. No residues were detected in apple juice and apple sauce (limit of determination, 0.01 mg/kg). The pomace may be used as animal feed (Ussary, 1977a, c; JMPR, 1980)

Tomato

As with apples, permethrin residues in whole tomatoes remain primarily in the pomace during processing. Permethrin levels in tomato juice, tomato puree and tomato ketchup were consistently much smaller than those found in whole tomatoes. The pomace may be used as animal feed (JMPR 1979). Residues in wet tomato pulp typically containing 25% dry matter were 10-50 (mean = 25) times the levels in whole tomatoes (Ussary, 1977d) (JMPR 1980).

Wheat

During the processing of treated whole wheat grain, permethrin residues are retained mainly in the bran component although a significant proportion (12%) remains with the white flour. Permethrin residues in flour from treated whole grain are carried over into bread baked from that flour; there is no reduction in residue level on a commodity dry-weight basis (Simpson, 1979). White flour retains about 12% of the whole grain residue. The major part of the residue, about 62%, remains with the bran and about 26% is in the pollard. Therefore, whilst white bread prepared from treated grain would have a residue of about 0.15-0.2 mg/kg, the corresponding level in whole meal bread would be about 0.7-1 mg/kg (Simpson, 1979). The processing operations simulated those used in commercial practice; the unbaked bread recipe included potassium bromate and benzoyl peroxide. Whole meal flour was formulated after reconstituting the wheat fractions in the original ratio.

FATE OF RESIDUES

Plants

The metabolic fate in plants has been investigated both in the field and under greenhouse conditions. The metabolic products from plants were identical with permethrin metabolites observed in mammals with the exception that glucose is the primary conjugating moiety. The major metabolites were products of ester cleavage (occurring in plants as well as mammals more rapidly with the *trans*- than the *cis*-isomer) and conjugation of the liberated acid and alcohol fragments. Minor oxidative pathways of both the acid and alcohol fragments have been identified.

The two important plant metabolites are 3-phenoxybenzyl alcohol and the *cis*- and *trans*-isomers of 3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylic acid (DCVA), also identified as animal metabolites. Levels of these metabolites in crops are much smaller than corresponding permethrin residues in food animals and they do not need to be included in routine plant residue analysis (JMPR, 1980).

In general, permethrin residues from foliar sprays are not translocated from site of deposition, nor is there any appreciable uptake into the aerial parts of plants from soils. Permethrin per se is relatively persistent on plant surfaces. On leaf surfaces, permethrin is degraded mainly by ester cleavage, occurring more rapidly with the *trans*-isomer than the *cis*-isomer. The major degradation products are the *cis*- and *trans*-isomers of 3-(2,2-dichlorovinyl) 2,2-dimethyl-cyclopropane carboxylic acid (DCVA) and 3-phenoxybenzyl alcohol (3-PBA), both free and as conjugates (Gatehouse et al., 1976a, b; Gaughan et al, 1976; Gaughan and Casida, 1978; Ohkawa et al, 1977; Selim and Robinson, 1977a, b).

The degradation of ^{14}C-permethrin has been studied on cotton leaves, bean seedlings, cabbage leaves and apple fruits. In all cases permethrin degraded comparatively slowly. Unchanged permethrin accounted for 23-58% of the radioactivity on cotton leaves after 28 days (Gatehouse et al, 1976b) more than 80% of the radioactivity in apple fruits after 28 days and more than 60% of the radioactivity on cabbage leaves after 42 days (Gatehouse et al, 1976b). On bean plants *trans*-permethrin was shown to degrade more readily than *cis*-permethrin with half-lives of 7 and 9 days, respectively (Gaughan and Casida, 1978; Ohkawa et al, 1977). Both isomers undergo ester cleavage and oxidation of the phenoxy group to produce the resulting acid and alcohol

Animals

Permethrin is extensively metabolized and rapidly excreted by cows and goats after oral administration. Residue levels in milk, muscle and fat are small, as they are in the skin, muscle and eggs of hens. Permethrin constitutes more than half of the residue in milk, eggs and fat, and in the muscle of livestock. In all cases, residue levels decline notably on cessation of exposure (JMPR 1980).

Cow

Groups of lactating cows were administered permethrin orally or dermally. Milk, blood and excretory products were analyzed for 7 or 14 days after which the animals were sacrificed for tissue analysis. Permethrin is rapidly absorbed by both routes of administration. Residue levels in the milk of both orally- and dermally- administered cows increased for 24 to 48 hours following administration, although following dermal administration, residues in milk were exceedingly low. Within 7 days all residues not detectable (Bewick and Leahey, 1976). In the animals dosed orally, 40% of the excreted radioactivity was found in the urine with 60% found in the faeces. Residue levels in adipose tissue were characterized as permethrin.

Lactating cows administered permethrin in ethanol orally at a dose of 1 mg/kg for three consecutive days had no adverse effect on the cows. At the end of a 12-days trial the animals were sacrificed and tissues and organs examined for residues. Permethrin was rapidly absorbed and excreted with the majority of residue, from 90-100% of the administered dose, recovered predominantly in urine and faeces. Milk and milk fat analyses were performed and small quantities of residues of both *cis*- and *trans*-permethrin (*cis*-isomer predominated) were observed, mainly in the lipid fraction. In general, there was a more rapid elimination of *trans*-permethrin and its metabolites than of *cis*-permethrin and its metabolites.

In general, the fat soluble permethrin isomers are rapidly metabolized and excreted by cows and goats (Gaughan, et al., 1978a; Hunt and Gilbert, 1977). In cows, permethrin is found in small quantities in milk fat and adipose tissue. Following multiple administration (3 days) to cows, the recovery of permethrin was nearly quantitative within 12 to 13 days.

Individual isomers of *cis*- and *trans*-permethrin were orally administered to lactating cows for three consecutive days at a dose rate of approximately 1 mg/kg body weight. Residues in milk consisted almost entirely of unmetabolized *cis*-permethrin. Trace levels of hydroxylated permethrin residues were also found in milk. The major excretory metabolites included hydroxylated permethrin (on the gem-dimethyl group), 3-phenoxybenzyl alcohol and a glutamic acid conjugate of 3-phenoxybenzoic acid. As noted with milk, most of the residues in adipose tissue were unmetabolized permethrin. In comparison with the metabolic profile observed in rats, cows excrete a larger proportion of ester metabolites, including their glucuronides, and are unique in utilizing glutamic acid for conjugation of the acidic metabolites. Quantitatively, cows carry out more extensive hydroxylation on the gem-dimethyl moiety and less on the benzoyl moieties resulting in a greater concentration of 4'-hydroxyphenoxybenzoic acid- (sulfate) metabolite in rats than in cows. Qualitatively, similar results to those noted with cows have been observed with goats (Hunt and Gilbert, 1977).

Goat

Goats were administered orally at a dosage rate of 20 mg/kg/day for 7 consecutive days. Low levels of residues were observed in the milk. The residue level appeared to plateau within 4-5 days of the initial treatment. A sample of milk, containing approximately 0.026 ppm in the whole milk, was analyzed for residues in milk fat. Fifty percent of the total residues was extracted from milk fat and was found to be unchanged permethrin although the cis:trans ratio changed from approximately 4:6 to 2:1 (Leahy, et al., 1977). At the conclusion of the study, low levels of residues were noted in various organs (i.e., kidney, liver and muscle) with extremely low levels in adipose tissue.

Chickens

Permethrin is absorbed, distributed, metabolized and excreted in hens with rates substantially faster than in mammalian species. Permethrin administered to laying hens for three consecutive daily doses of 10 mg/kg was rapidly absorbed and distributed, being eliminated within one day after the final dose. Approximately 90% of the administered dose was recovered in excreta with small residues noted in eggs (predominantly yolk) and in adipose tissue. The residue observed in hen was predominantly the *cis*-isomer.

The metabolic fate in hens was investigated following oral administration of a dose of 10 mg/kg/day for three consecutive days. The overall metabolic pathway was similar to that noted with mammalian species. Permethrin was effectively hydrolyzed and oxidized with the *trans*-isomer being metabolized more extensively. In egg yolk, permethrin and *trans*-hydroxymethyl *cis*-permethrin were detected as residues. Extensive metabolism via hydrolytic, oxidative and conjugative reactions is probably responsible for the relative insensitivity of permethrin in avian species (Gaughan, et al., 1978b).

METHODS OF ANALYSIS

The preferred method of permethrin residue analysis in crops is by gas chromatography using an electron capture detector. Recoveries are essentially quantitative and the method has been applied successfully to a wide range of crops, raw cereal grains and processed products derived from them, such as flour, bran and bread. A limit of determination of 0.01 mg/kg (expressed as permethrin *cis* and *trans*-isomers). *Cis* and *trans*-isomers are capable of being determined separately by this method. With small modifications, the method has been applied successfully to the determination of permethrin residues in meat, milk and eggs, and with adaptation, may be suitable for regulatory purposes. GLC/mass spectrometry was cited as a procedure for qualitative and quantitative estimation of residue.

The free and conjugated metabolites DCVA and 3-PBA can also be determined by GLC/EC after derivatization. Conjugates are freed by refluxing in acid and determined as the 2,2,2-trichloroethyl ester of DCVA and the heptafluorobutyl ester of 3-PBA. The lower limits of detection are reported to be 0.02-0.10 mg/kg for DCVA and 0.02-0.05 mg/kg for 3-PBA (depending on substrate).

APPRAISAL

In pre harvest uses, residues are highest where a crop part is exposed directly to the spray e.g. forage crops. Ground and aerial application yield similar residue levels. Although residues decline comparatively slowly after spraying, there is no obvious build-up of residues of permethrin or its two most important plant metabolites on repeated application, within the rates and frequency of permethrin spraying that are needed to obtain good insecticidal control. The two important plant metabolites are 3-phenoxybenzyl alcohol and the *cis*- and *trans*-isomers of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (DCVA). These are also animal metabolites. The amounts of these metabolites in crops are very much smaller than permethrin residues and they do not need to be included in routine residue analyses (JMPR, 1980).

Permethrin levels in stored grains decline slowly. During the processing of treated whole wheat grain, the permethrin residue is retained mainly in the bran component, although a significant proportion (12%) remains with the white flour and follows through into bread baked from that flour.

After both oral and dermal administration to livestock, the small amounts of permethrin constitute the major portion of the residue in milk, eggs, muscle and fat. After oral administration to goats, metabolites form the major part of the residue in kidney and liver; DCVA occurs in both organ tissues, 3-phenoxybenzyl alcohol plus its 4'-hydroxy derivative, in liver and 3-phenoxybenzoic acid plus its 4-hydroxy derivative, in liver and kidney.

Following direct application to dairy cattle (1.0 g ai/cow and with free access to a self-oiler) permethrin levels are low in milk (<0.02 mg/kg) and in muscle, liver and kidney (<0.01 mg/kg). Highest levels (up to 0.1 mg/kg) have been found in fat.

Cows maintained on a diet containing 50 mg/kg of permethrin yielded milk containing low levels of residue (0.1 mg/kg), and the level in muscle was less than 0.1 mg/kg. The residue in milk declined to below 0.01 mg/kg after five days on returning the cows to a control diet.

Residues in eggs are also low and contamination of eggs laid during the treatment of poultry houses will not result in detectable residues in yolk and albumen. Permethrin is not detected in the albumen of eggs from hens receiving the compound at levels up to 33 mg/kg in the diet; levels in yolk are approximately 2% of corresponding permethrin dietary levels. In all cases studied, residues of permethrin and its metabolites in products of animal original declined notably on cessation of exposure.

Only small residue levels have been found in products of animal origin following direct application of permethrin to livestock. In view of the levels of permethrin found in forage crops, such as alfalfa, and the importance of such crops in animal feedstuffs, it seems likely that residue levels in products of animal origin will be higher following forage crop uses than from use for ectoparasite control (JMPR 1980).

MAXIMUM RESIDUE LIMITS

Codex MRLs have been established for a variety of fruits and vegetables. Feed commodities include alfalfa fodder, apple pomace, maize fodder, sorghum straw and fodder (dry) and soya bean fodder. MRLs for animal products include: edible offal (mammalian), eggs, meat (from mammals other than marine mammals), milks and poultry meat. The MRLs are summarised in Table 1.

Table 1. Permethrin MRLs in plant and animal products

Main uses	8 INSECTICIDE
JMPR	79, 80R, 81, 82R, 83R, 84R, 85R, 86, 87T, 88R, 89R, 91R (99T')
ADI	0.05 mg/kg body weight (1987)
RESIDUE	Permethrin (sum of isomers) (fat-soluble) ADI applies to the nominal 40% cis-, 60% trans- and 25% cis- 75% trans- materials only

Code	Commodity Name	MRL (mg/kg)	Step	JMPR	CCPR
AL 1020	Alfalfa fodder	100 dry wt	CXL		
TN 0660	Almonds	0.1	CXL		
AB 0226	Apple, pomace, Dry	50	CXL		
VS 0621	Asparagus	1	CXL		
VD 0071	Beans (dry)	0.1	CXL		
FB 0264	Blackberries	1	CXL		
VB 0400	Broccoli	2	CXL		
VB 0402	Brussels sprouts	1	CXL		
VB 0403	Cabbage, Savoy	5	CXL		
VB 0041	Cabbages, Head	5	CXL		
VR 0577	Carrot	0.1	CXL		
VB 0404	Cauliflower	0.5	CXL		
VS 0624	Celery	2	CXL		
GC 0080	Cereal grains	2 Po	CXL		
VL 0467	Chinese cabbage (type petsai)	5	CXL		
FC 0001	Citrus fruits	0.5	CXL		
SB 0716	Coffee beans	0.05	CXL		
VP 0526	Common bean (pods and/or immature seeds)	1	CXL		
SO 0691	Cotton seed	0.5	CXL		
OR 0691	Cotton seed oil, Edible	0.1	CXL		
VC 0424	Cucumber	0.5	CXL		
FB 0021	Currants, black, red, white	2	CXL		
FB 0266	Dewberries (including boysenberry and loganberry)	1	CXL		
MO 0105	Edible offal (mammalian)	0.1 V	CXL		
VO 0440	Eggplant	1	CXL		

Code	Commodity Name	MRL (mg/kg)	Step	JMPR	CCPR
PE 0112	Eggs	0.1	CXL		
VC 0425	Gherkin	0.5	CXL		
FB 0268	Gooseberry	2	CXL		
FB 0269	Grapes	2	CXL		
DH 1100	Hops, Dry	50	CXL		
VR 0583	Horseradish	0.5	CXL		
VL 0480	Kale	5	CXL		
FI 0341	Kiwifruit	2	CXL		
VB 0405	Kohlrabi	0.1	CXL		
VA 0384	Leek	0.5	CXL		
VL 0482	Lettuce, head	2	CXL		
AS 0645	Maize fodder	100 dry wt	CXL		
MM 0095	Meat (from mammals other than marine mammals)	1 (fat) V	CXL		
VC 0046	Melons, except watermelon	0.1	CXL		
ML 0106	Milks	0.1 (fat)	CXL		
VO 0450	Mushrooms	0.1	CXL		
FT 0305	Olives	1	CXL		
SO 0697	Peanut	0.1	CXL		
VP 0064	Peas, shelled (succulent seeds)	0.1	CXL		
VO 0051	Peppers	1	CXL		
TN 0675	Pistachio nuts	0.05	CXL		
FP 0009	Pome fruits	2	CXL		
VR 0589	Potato	0.05	CXL		
PM 0110	Poultry meat	0.1	CXL		
VR 0591	Radish, Japanese	0.1	CXL		
SO 0495	Rape seed	0.05	CXL		
FB 0272	Raspberries, red, black	1	CXL		
AS 0651	Sorghum straw and fodder, dry	20	CXL		
VD 0541	Soya bean (dry)	0.05	CXL		
AL 0541	Soya bean fodder	50 dry wt	CXL		
OC 0541	Soya bean oil, crude	0.1	CXL		
VL 0502	Spinach	2	CXL		
VA 0389	Spring onion	0.5	CXL		
VC 0431	Squash, Summer	0.5	CXL		
FS 0012	Stone fruits	2	CXL		
FB 0275	Strawberry	1	CXL		
VR 0596	Sugar beet	0.05	CXL		
SO 0702	Sunflower seed	1	CXL		
OC 0702	Sunflower seed oil, crude	1	CXL		
OR 0702	Sunflower seed oil, Edible	0.1	CXL		
VO 0447	Sweet corn (corn-on-the-cob)	0.1	CXL		
DT 1114	Tea, green, black	20	CXL		
VO 0448	Tomato	1	CXL		
CM 0654	Wheat bran, unprocessed	5 PoP	CXL		(1993)
CF 1211	Wheat flour	0.5 PoP	CXL		(1993)
CF 1210	Wheat germ	2 PoP	CXL		(1993)
CF 1212	Wheat wholemeal	2 PoP	CXL		(1993)
VC 0433	Winter squash	0.5	CXL		

REFERENCES

Bewick, D.W. and Leahey, J.P. (1976). Permethrin: Absorption in cows. ICI Plant Prot. Div. Report No. TMJ1357B (unpublished).

Gatehouse, D.M., Leahey, J.P. and Carpenter, P.K.(1976b). Permethrin degradation on cotton. ICI Plant Prot. Div. Report No, AR2701B (unpublished).

Gaughan, L.C., Unai, T. and Casida, J.E.(1976). Permethrin metabolism in rats and cows, and in bean and cotton plant. Paper delivered at 172nd ACS National Meeting, San Francisco (August 1976).

Gaughan, L.C. and Casida, J. E. (1978). Degradation of *trans-* and *cis-* permethrin in cotton and bean plants, J. Agric. Food Chem., 26, (3), 525-8.

Gaughan, L.C., Robinson, R.A and Casida, J.E. (1978b). Distribution and metabolic fate of *trans* and *cis-* permethrin in laying hens, J. Agric. Food Chem., 26 (6), 1374-1380.

Glenn, M.S. and Sharpf, W.G. (1977). American Chemical Society, Symposium Series 42, 116.

Hunt, L.M. and Gilbert, B.N. (1977). Distribution and excretion rates of ^{14}C-labelled permethrin isomers administered orally to four lactating goats for 10 days, J. Agric. Food Chem., 25 (3), 673.

Ohkawa, H., Kaneko, H. and Miyamoto, J. (1977). Metabolism of permethrin in bean plants. J. Pesticide Sci., 2, 67-76.

Selim, S. and Robinson, R.A.. (1977). Uptake of permethrin by cotton plants. No. M-4099; Degradation on Cotton leaf. M-4118. FMC Report (unpublished).

Simpson, B.W. (1979). Queensland Department of Primary Industries, Agricultural Chemistry Branch, Queensland, Australia (unpublished data).

Ussary, J.P. (1977a). Permethrin residues in the commercial processing fractions of apples, Wenatchee, Washington, 1976. ICI Americas Inc. Report No. TMU0383/B (unpublished)

Ussary, J.P. (1977c). Permethrin residues in the commercial processing fractions of apples, Geneva, New York, 1976. ICI Americas Inc. Report No. TMU0307/B (unpublished).

Ussary, J.P. (1977d). Permethrin residues in tomato process fractions. ICI Americas Inc. Report No. TMU0304/B (unpublished).

Ussary, J.P. and Braithwaite, G.B.(1980a). Residues of permethrin and permethrin metabolites in milk from ECTIBAN treated cows (Trial No. 35NC79-001). ICI Americas Inc. Report No. TMU 490/B (unpublished).

Ussary, J.P. and Braithwaite, G.B. (1980b). Residues of permethrin and 3-phenoxy-benzyl alcohol in cow tissues (Trial No. 35NC79-001). ICI Americas Inc. Report No. TMU0493/B (unpublished).

Ussary, J.P. and Braithwaite, G.B. (1980c). Residues of permethrin and 3-phenoxybenzyl alcohol in tissues from ECTIBAN treated swine (Trial No. 35NC79-002). ICI Amercas Inc. Report No. TMU 0491/B (unpublished).

Ussary, J.P. and Braithwaite, G.B. (1980d). Residues of permethrin and 3-phenoxybenzyl alcohol in tissues and eggs from ECTIBAN treated chickens (Trial No. 35NC79-003). ICI Americas Inc. Report No. TMU0492/B (unpublished).

TRICHLORFON
(METRIFONATE)

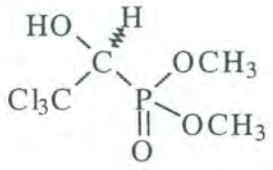
First draft prepared by
Raymond Heitzman, Newbury, United Kingdom
Jose Martinez, San Jose, Costa Rica
Robert Wells, New South Wales, Australia

IDENTITY

Chemical Name: Dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate (IUPAC & CAS name); CAS No. 52-68-6

Synonyms: Trichlorfon, Metrifonate

Chemical Structure:



Molecular formula: $C_4H_8Cl_3O_4P$
Molecular weight: 257.45

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: A racemic mixture of *cis*- and *trans*- Trichlorfon
Appearance: White crystals
Solubility: Water, 13.6; methanol, 107; ethanol, 87.6; acetone, 63.1; acetonitrile, 69.4; n-heptane, 0.15 (all in g/100ml at 25°C)
Melting point: 76-81°C
Stability: No information provided

RESIDUES IN FOOD AND THEIR EVALUATION

Introduction

The product is used for cattle and horses (as minor species). Trichlorfon is an old drug and a large part of the data was generated before the introduction of GLP. Nevertheless there is a substantial amount of new information provided that was mainly done in accord with GLP.

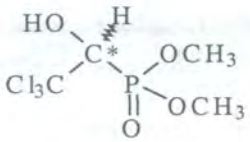
Conditions of use

Trichlorfon is an organophosphorus compound with insecticidal, acaricidal, and anthelmintic properties. It is used orally, topically or parentally for the control of parasites in various animal species. Humans may be treated orally with trichlorfon for infestations of *Schistosoma haematobium*, and it has some use in the treatment of Alzheimer disease. Trichlorfon is used as an insecticide on food crops and forests.

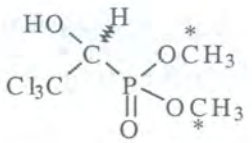
Cattle are treated orally or with aqueous pour-on, wash, or spray solutions at 50–75 mg/kg of body weight. Repeated dosing may be necessary. The preparations for use on horses are similar, but the oral dose is 35 mg/kg of body weight; one topical application for use on horses contains febantel.

PHARMACOKINETICS

Trichlorfon used in the pharmacokinetics studies was labelled in one of two carbons with ^{14}C as shown below.



^{14}C ethyl trichlorfon



^{14}C methyl trichlorfon

Laboratory Animals

Rat

[Ethyl-^{14}C]-trichlorfon was administered to groups of 5 male albino rats in a single dose of 10 mg/kg either orally, i.v., or intraduodenally. Additionally [methyl-^{14}C]-trichlorfon was administered orally at 10 mg/kg to 5 male rats. Pharmacokinetic measurements were made over a 48 hour period (Ahr and Siefert, 1992). In a second study rats weighing about 200 g were dosed orally either once or repeatedly for 21 consecutive days with 10 mg/kg BW [ethyl-^{14}C]-trichlorfon (Schwarz et al, 1994). Both studies were not compliant with GLP.

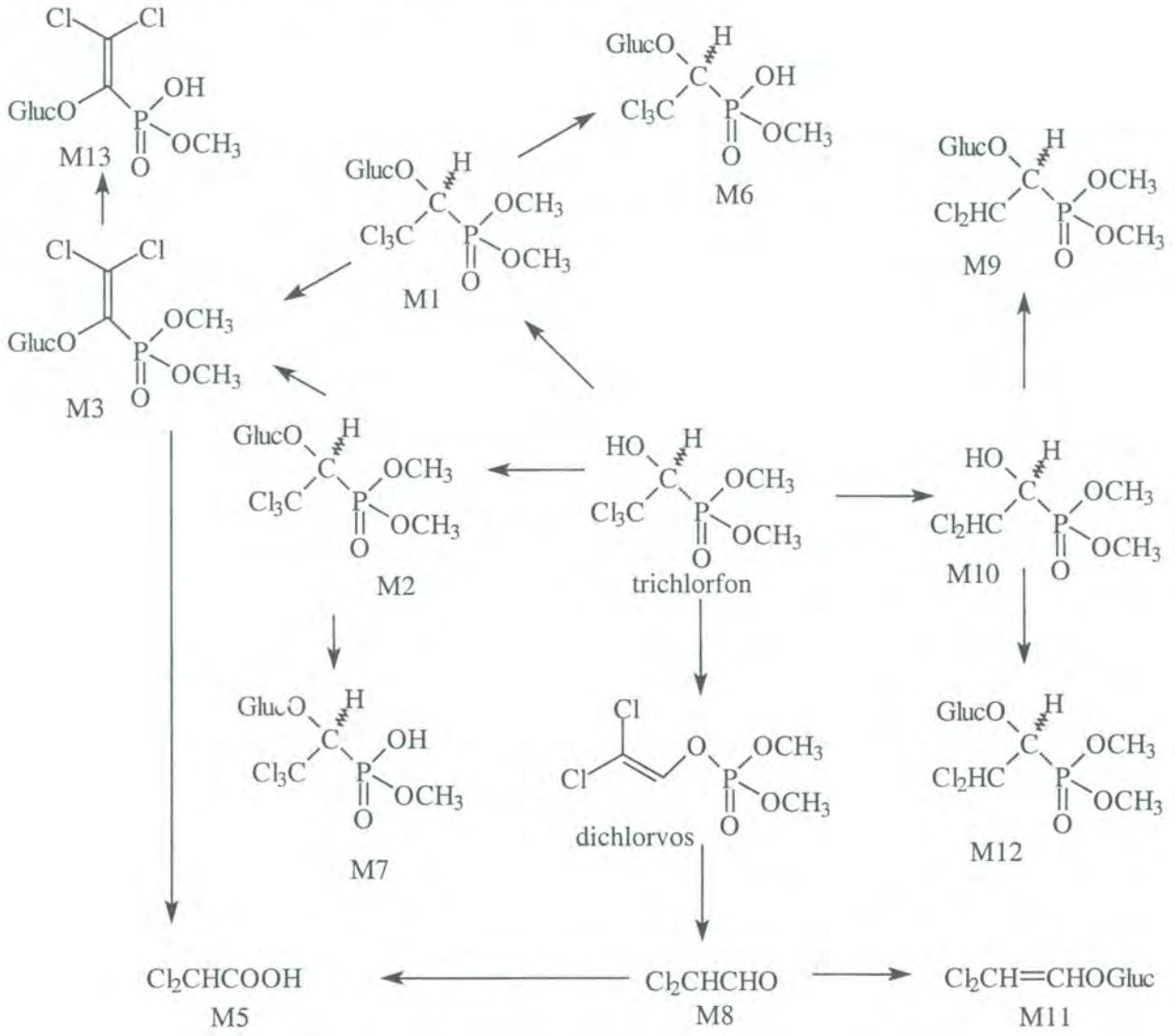
Absorption
The drug was rapidly absorbed following oral administration and reached peak concentrations in plasma usually in <1 hour. There were higher values for the AUC in the earlier 1992 study than in the 1994 study. Otherwise similar values for the parameters were obtained. After 21 repeat doses the t_{max} was the same (0.5 h) but other parameters were obviously increased; e.g. C_{max} = 10.4 mg/L; $t_{1/2}$ = 28.4 h and AUC = 494 mg.h /kg.

Elimination
The radiolabelled trichlorfon was rapidly excreted into expired air, urine and faeces (Ahr and Siefert, 1992; Schwarz et al., 1994). The results are shown in table 1. The study also included a group of four rats with biliary cannulae and analysis of the data showed that there was clear evidence of enterohepatic recirculation following intraduodenal administration of 10 mg/kg BW [ethyl-^{14}C]-trichlorfon. Because of the extensive metabolism of the drug, the expired $^{14}CO_2$ was high (20-26% dose) and similar for both the different routes of administration or the different labelling position of the ^{14}C. Of the radioactivity not expired as $^{14}CO_2$, 43-54% of the dose was excreted in the urine and about 20% in the faeces. When repeated oral doses were administered, 39% and 38% of the total dose was excreted in the urine and faeces, respectively.

Metabolism
More than 20% of the dosed radioactivity is expired as $^{14}CO_2$ (Ahr and Siefert, 1992). This indicates extensive and rapid metabolism in rats. Metabolites were identified in the plasma, urine and bile by Boberg et al., (1993) using HPLC, MS, NMR, GC-MS and LC-MS.. They are: rearrangement to form dichlorvos, that is further metabolised to dichloroacetaldehyde and then dichloroacetic acid; replacement of a Cl by H to form the $CHCl_2$ group; formation of a glucuronide at the OH group; demethylation of the OCH_3 group to an OH group;.the two diastereoisomers, M1 and M2, are converted to M3 by removal of HCl; M3 is further metabolised to either M13 or dichloroacetic acid.

The metabolites except dichlorvos and parent drug, are assigned M numbers similar to those used by the sponsor. The diastereoisomers, M1 and M2 formed from trichlorfon and M9 and M12 formed from M10 have been identified. The biotransformation to M1 and M2 and further to M3 was a major pathway, however more M1 isomer than M2 isomer was found in plasma and urine indicating stereoselective glucuronidation. Metabolites M1, M2 and M3 were the most abundant metabolites. The main trichlorfon biotransformations are shown in Figure 1below.

Figure 1. Biotransformation of Trichlorfon in the rat.



Rabbits

Two complementary non-GLP compliant studies were carried out using rabbits. In both studies (Ahr et al., 1993, 1994), three NZ white rabbits were administered [ethyl-^{14}C]-trichlorfon as a single oral dose of 67 mg/kg BW. Three rabbits also received a single i.v. bolus of dichlorvos (Ahr and Zimmer, 1994). Pharmacokinetic parameters were determined for blood plasma and excreta using the total radioactivity or the concentrations of the (+) and (-) enantiomers of trichlorfon.

Absorption

The absorption of radiolabelled drug was rapid and complete (>99%). The peak concentrations of total radioactivity for the (-) trichlorfon isomer and the (+) trichlorfon isomer were 102, 16 and 1 mg/l plasma, respectively, and attained at 30, 15 and 25 min, respectively.

Elimination

[Ethyl-^{14}C]-trichlorfon was almost completely eliminated through the urine (99% of the dose) with only 1% of the dose in the faeces. This varies from the rat in that there is no evidence that the radioactivity would be expired as $^{14}CO_2$.

Metabolism

The metabolites found in both plasma and urine were broadly similar to those found in the rat (see table 2). The exceptions were the absence of parent drug in the rabbit plasma and urine and the presence of M13 (8.5% dose) in the urine. Ninety-three percent of the initial dose of radioactivity was accounted for in the metabolite profiles for rabbit urine, whereas only 53% was accounted for in rat urine.

Monkey

A GLP compliant study used three female Rhesus monkeys dosed orally with 5 mg/kg BW [ethyl-^{14}C]-trichlorfon. The same animals were administered the same i.v. dose in a cross-over design experiment (Cornelissen, 1993). The biotransformation of trichlorfon was carried out in a non-GLP compliant study by Boberg et al. (1994), using plasma and urine samples from the above study.

Absorption
The absorption of radiolabelled drug following oral dosing was rapid and almost complete (>83% of the dose). Peak plasma concentration was 6.8 mg.eq/l reached at 1 hour post dosing. In both treatments, radioactivity was initially rapidly eliminated, with an approximate.$t_{1/2}$ ~1.2 h., followed by a terminal elimination with a half-life of about 100 h.

Elimination
Radioactivity was excreted in the urine (> 75% of the dose) and was almost complete with 4 hours. 6.8% of the dose was in the expired air and only 2.3% in the faeces.

Metabolism
As found in the rat and rabbit, the major urinary metabolites were the enantiomers M1 and M2 and their degradation product, M3. Other metabolites were the same as for the rat with the exception of M14, a dichloroethanol glucuronide, a minor component. M14 is formed by the following route:

Trichlorfon → dichlorvos → dichloroacetaldhyde (M8) → dichloroethanol (M4) → dichloroethanol-glucuronide (M14).

Results of the pharmacokinetics and metabolites are shown in tables 1 and 2, respectively.

Table 1. Plasma pharmacokinetic parameters and excretion of ^{14}C measured as ^{14}C-trichlorfon equivalents in rats and in rabbits.

	i.v. ethyl-^{14}C-trichlorfon	Oral ethyl-^{14}C-trichlorfon	Oral ethyl-^{14}C-trichlorfon	Oral methyl-^{14}C-trichlorfon	Oral ethyl-^{14}C-trichlorfon	Oral [ethyl-^{14}C]-trichlorfon	
						(-) isomer	(+) isomer
Pharmacokinetic Parameters							
Study	Rat (1992)	Rat (1992)	Rat (1994)	Rat (1992)	Rabbit (1993)	Rabbit (1994)	Rabbit (1994)
	105 ± 1.1	98 ± 1.2			350 ± 1.1[d]		
	158 ± 1.1	138 ± 1.3	69 ± 1.3		536 ± 1.1	14.5 ± 1.2	0.73 ± 1.5
	15.8	13.8	6.9		8.24	0.43	0.02
	1.21 ± 1.34				0.78 ± 1.2[c]		
	33.5 ± 1.1	27.7 ± 1.2	15.4 ± 1.1		64.4 ± 1.3	0.49 ± 1.53	0.85 ± 2.46
C_{max} (mg/L)	-	5.44 ± 1.1	4.6 ± 1.2	4.4 ± 1.1	102 ± 1.4	16.1 ± 1.5	0.93 ± 2.1
t_{max}(h)	-	0.51 ± 1.5	0.58 ± 1.37	0.33 – 0.67	0.5 ± 1.5	0.26 ± 2.22	0.42 ± 1.49
Excretion Characteristics							
Expired $^{14}CO_2$	20.3 ± 5.2[a]	20.6 ± 2.1[a]	not measured	25.9 ± 3.0[a]	not measured	not measured	Not measured
Urine (% dose)	47.4 ± 8.0[a]	46.1 ± 3.6[a]	42.9 ± 0.8[b]	54.4 ± 3.1[a]	99[d]	not measured	Not measured
Faeces (% dose)	21.9 ± 5.8[a]	21.2 ± 3.2[a]	19.6 ± 2.4[b]	9.1 ± 1.0[a]	1[d]	not measured	Not measured

Note. Pharmacokinetic parameters are geometric means ± SD; excretion characteristics are mean ± SD. The dose in rats is 10 mg/kg BW (n = 5); in rabbits, 67 mg/kg BW (n = 3). The (+) and (-) enantiomers were quantitated separately. The results for rats are taken from Ahr & Siefert (1992), Schwarz et al. (1994a); for rabbits, Ahr and Zimmer, (1994) and Ahr, et al., (1993)
Footnote. [a] measured over 48 hours, [b] measured over 24 hours, [c] measured over 1-3 h, [d] measured over 0-72 h.

Table 2. Metabolites of trichlorfon as percent radioactivity in plasma and as percent dose in the urine in test animals after an oral dose of [ethyl-^{14}C]-trichlorfon and in one cow after an oral dose of ^{32}P-trichlorfon.

Species	Rat		Rabbit		Monkey		Cattle	
Compound	Plasma 0.66h 5h	Urine 24h	Plasma 0.75h 1.5h	Urine 24h	Plasma 0.75h 1.5h	Urine 24h (n=2)	Blood 2 h 3h	Urine 12h
Trichlorfon	6.4 (-)	0.3			3.2 2.3	0.8	7.5 6.8	0.2
Dichlorvos						0.04		n.d.
M1	29.0 (-)	9.1	23.1 14.2	5.1		18.7		
M2	11.4 (-)	7.0	3.1 2.0	1.6		4.5		
M3	41.3 (-)	14.4	49.5 48.1	59.7	70.9 57.9	29.7		50
M4					2.1^{t} 2.2^{t}			
M5	1.3 5.0				8.4 10.7	0.4		
M6		0.8		0.2		1.0		
M7		1.0		0.6		4.6		
M8					2.2 3.4	2.8		
M9	3.1 (-)	0.4		0.4				
M10		1.0		see M9				
M11	see M9			see M9		3.4		
M12		0.3		0.2		0.3		
M13				8.5		2.4		
Others not identified	8.7 91.0	18.7	9.5 15.2	16.5	(-) (-)	3.9		15.8
% dose		52.9		92.8		73.3		66.0

Note. (-) is not found. See M9 means that compound was identified but co-eluted with M9. Footnote: ^{t} is tentative result

Food Producing Animals

Cattle: Topical Administration

The radiolabelled drug applied topically in the cattle studies was [ethyl-^{14}C]-trichlorfon. The purity was 98.6 - 98.7% and the specific activity was 2.11 GBq/mmol. The [ethyl-^{14}C]-trichlorfon was applied to the back of 8 calves at a target dose level of 40 mg /kg BW. The heads of the animals were restrained throughout the study period to prevent oral ingestion of the dose (Lynch and Speirs, 1998).

Absorption
14-49% of the dose was not available for absorption because of run off following application. Absorption of the test material through the skin was assessed by measuring total radioactivity in plasma at various times post dose and by measuring daily excretion of radioactivity in urine and faeces until 120 h post dose in two of the calves. Absorption into the systemic circulation was rapid with the maximum plasma concentration of total radioactivity observed at 4 h post dose in males and 6 h in females. Plasma concentrations of total radioactivity at this time (Cmax) represented 1.01 mg equiv./kg and 0.36 mg equiv./kg, in the two calves, respectively. Results indicate that plasma concentrations of total radioactivity declined in a biphasic manner, rapidly to 24 h and from 24 -120 h post dose thereafter more slowly. The plasma elimination half-life of total radioactivity over this period represented 124 h and 258 h in the two calves, respectively.

Elimination
Over 0-120 h post dose, excretion of total radioactivity was relatively low in the two calves, with 2.8% and 1.6% of the administered dose excreted in urine, and 3.3% and 0.3% of the administered dose in the faeces of the two calves, respectively. Taking into account that a significant portion of the administered dose was not available for absorption due to run-off, then the amount excreted as a percentage of the retained dose would be higher. Over 120 h post dose, 13.6% and 5.2% of the retained dose was excreted in urine and 16.2% and 1.0% of the retained dose in faeces of the two calves, respectively.

Metabolism

The tissues from the calves slaughtered on 1day post-dosing were investigated for metabolic profiles (Phillips and Johnson, 1998). Samples of muscle, liver and kidney were acidified with phosphoric acid and sequentially extracted with distilled water; acetonitrile; acetonitrile:water and 1% acetic acid in methanol. The non-extracted portion was extracted further following hydrolysis with a pepsin/HCl mixture. Fat was acidified with phosphoric acid and sequentially extracted with acetonitrile; acetonitrile:water and 1% acetic acid in methanol. The non-extracted portion was further extracted with acetone:acetonitrile and finally with acetonitrile:NaOH. The tissues collected for the calves killed on days 2, 3 and 5 were similarly evaluated (Phillips, 1998). The total residues (TR) and the percentage of TR that were extractable are shown in Table 3.

Table 3. The total residues and their extractability in tissues of calves treated with a pour-on preparation of [ethyl-^{14}C]-trichlorfon.

Tissue	Total Residues as µg eq./kg with percent extractable (in parentheses)			
	1 day [a]	2 day [c]	3 day [c]	5 day
Muscle distant	20-305 (90)			
close	488, 687 (88)			
Muscle Composite		218 (96)	185 (90)	92, 92 (94)
Liver	396, 779 (96)	1272 (83)	1947 (91)	824, 1056 (90)
Kidney	309, 622 (96)	732 (91)	1153 (92)	548, 579 (97)
Fat Renal	21, 23 (80)			
Omental	65, 18 (78)			
S.c. distant	84, 139 (86)			
S.c. close	1054, 882 (85)			
Fat Composite		807 (78)	950 (73)	119, 274 (78)

Note. Distant or close indicates samples were collected either distant from or close to the dose area. Composite muscle and fat included tissues distant and close to dosing area.

Footnote. [a] TR in individual animals with mean value for extraction; [c] composite tissue of two animals.

The extractable fractions were used for metabolite identification. This was difficult due to the complex nature of the residues, the large amount of polar material and several minor metabolites. In fat, trichlorfon and dichloroacetic acid were identified as major metabolites in all the day 1 samples. Dichlorvos was found in one of the day 1 fat samples. Liver and kidney contained mainly polar metabolites and other unknown metabolites. Muscle contained trichlorfon and a compound provisionally identified as desmethyl-dichlorvos. It was not possible to identify a ratio of a single compound, e.g. trichlorfon, to the total residues.

Cattle: Oral administration

A grub-infested, lactating dairy cow was administered ^{32}P labelled trichlorfon as an oral dose of 25 mg/kg BW (Robbins et al., 1956). Samples of blood, urine, faeces and milk were collected. The concentrations of total radioactivity, trichlorfon and dichlorvos were measured. Peak blood concentration (Cmax = 15.1 mg equiv./kg) was attained at 2 h post dosing of which 7.5% was trichlorfon. The trichlorfon was mostly eliminated (66% within 12 hours) in the urine. Only 0.26% of the radioactivity excreted was trichlorfon. A major metabolite accounted for 77% of the radioactivity excreted and was tentatively identified as M3. Less than 3% of the radioactivity was excreted in the faeces. Dichlorvos was not detected in any of the samples.

Goats

The metabolic fate of [ethyl-^{14}C]-trichlorfon following oral administration of a single dose of 8.56 mg/kg BW on three consecutive days was studied in two lactating goats (Chopade *et al.*, 1987). The goats were sacrificed 4 hours after the last dose and samples of tissues and milk were collected and subsequently analysed for their metabolite composition. Unmetabolised trichlorfon was detected in small amounts (6-7%) in muscle and kidney but was not present in liver, fat or milk. A large proportion of the radioactivity was incorporated into tissue proteins and sugars and accounted for 38%, 52% and 23% or the Total Residues in muscle, liver and kidney, respectively. The conjugates of M5 (see figure 1) accounted (as %TR) for 43% in muscle, 11% in liver, 44% in kidney and 70% in fat. Other metabolites, namely, desmethyltrichlorfon, desmethyldichlorvos, M1, M2 and M4, were identified at low levels. The metabolic pathways proposed for the goat have some similar pathways to those of the rat but other routes are different.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

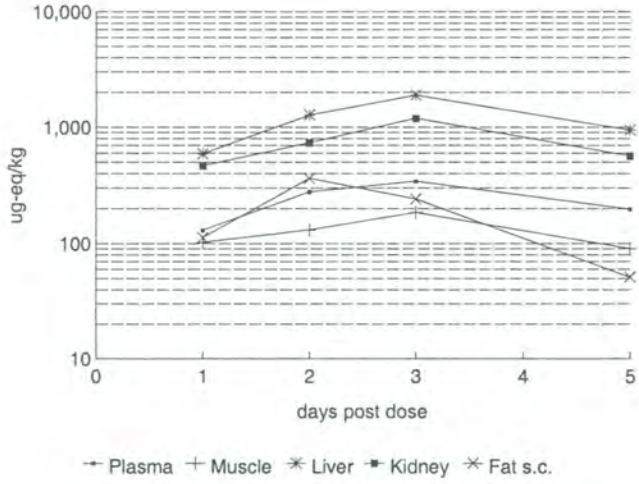
Cattle. Topical administration

[Ethyl-^{14}C]-trichlorfon was applied to the back of 8 calves at a target dose level of 40 mg /kg BW. The heads of the animals were restrained throughout the study period to prevent oral ingestion of the dose (Lynch and Speirs, 1998). One male and one female were sacrificed at each of 1, 2, 3 and 5 days post-dosing. Edible tissue samples and plasma samples were collected, stored at –20°C, and subsequently analysed for residues. Samples of fat, muscle and skin, close to and distant from the dose area were collected for analysis.

In the first few hours after dosing a large proportion (14-49%) of the dose ran off the animals and was not available for absorption. At slaughter, the animals were washed and the radioactivity in the washings was 4 -16% of the dose. The amount of dose still remaining on the skin was 8-28% and would continue to contribute to the tissue residues as the drug was absorbed. Only 2 and 6% of initial dose was estimated as absorbed in the two calves slaughtered at 5 days post-dosing, respectively.

The amount of radioactivity in plasma and tissues distant from the dose area reached a maximum 3 days and are depleting by five days post-dosing (see table 3 and figure 2). This may be due to the large part of the dose remaining on the skin - an essential requirement for prolonged ecto-parasiticidal activity.

Figure 2. Total residues (µg trichlorfon equiv./kg) in plasma and edible tissues of calves after dosing with a pour-on preparation of [ethyl-^{14}C]-trichlorfon at 40 mg/kg BW



Note. The values are the means for 2 calves. The values for muscle are the means for the combined values for both flank and round muscle.

Cattle. Oral administration

A grub-infested, lactating dairy cow was administered ^{32}P labelled trichlorfon as an oral dose of 25 mg/kg BW (see above) (Robbins et al., 1956). Samples of milk were collected. The concentrations of total radioactivity were measured. Peak milk concentration (Cmax = 2.3 mg equiv./kg) was attained at 18 hours post dosing of which only traces were identified as trichlorfon. Dichlorvos was not detected in any of the samples. The milk samples were subjected to bioassays to determine insecticidal activity to house flies. None of the samples had insecticidal activity, suggesting that the metabolic products were not toxic to insects.

Other residue depletion studies with unlabeled drug

Cattle

Numerous residue depletion studies using unlabelled drug administered either orally or topically were performed during 1959-69 (before GLP). The following cattle studies were submitted: Auer and Krebber (1998) and Aur et al., (1999) and 4 older non-GLP studies. The analytical methods used were not generally specific for trichlorfon or dichlorvos. Residue determination using the inhibition of cholinesterase enzyme activity would have been of particular interest. It may be possible to correlate the inhibition of cholinesterase activity with later results where it is certain that no residues of trichlorfon and dichlorvos and perhaps no biological activity are found.

Two new GLP compliant studies were submitted by the sponsors. The residues of trichlorfon and dichlorvos were measured in tissues and milk of cattle after topical application of unlabelled trichlorfon (Auer and Krebber, 1998). Sixteen German Pied adult cattle (8 male and 8 female) were treated with a single spray of 5% solution of trichlorfon at a dose rate of 40 mg trichlorfon/kg B.W. Groups of 2 males and 2 females were slaughtered at 12 hour, 1, 3 and 7 days after treatment. Liver and kidney samples were collected. Samples of muscle and fat were collected at sites distant and close to the area of application. The samples were analysed for residues of both trichlorfon and dichlorvos by a validated LC-MS/MS method (Krebber, 1998). No residues of trichlorfon were detected (LOD = 50 µg/kg) in muscle and fat distant from the dosing area nor in liver and kidney. No residues (LOD = 50 µg/kg) of dichlorvos were found in any tissue except in fat tissue close to the dose site taken from one animal at day 1. Samples of muscle and fat from this animal also contained residues of trichlorfon. The results for muscle and fat close to the dosing area are shown in Table 4. 12 hours after dosing residues were found in the subcutaneous fat close to the dose area in all four animals.

In the second study 8 dairy cows were treated with a single spray of a 5% solution of trichlorfon at a dose rate of 40 mg trichlorfon/kg B.W. (Auer et al., 1999). Four cows had high milk yields and four had low yields. Milk was collected and the concentration of trichlorfon measured by a validated LC-MS/MS method (Krebber, 1999). Milk samples were collected up to seven days post treatment. The results are shown in table 4. Trichlorfon, was excreted in the milk, primarily during the first 12 hours post-dosing. The highest level was 205 µg/kg in one cow sampled at 6 h post-dose. Residues were present just above the LOQ (25 µg/kg) in one cow at 24 hours and another cow at 36 hours but thereafter no residues were detected (LOD 2.5 µg/kg).

Table 4. Residues (µg/kg) of trichlorfon and dichlorvos in cattle tissues after a 40 mg/kg B.W. pour-on treatment of trichlorfon.

TISSUE and RESIDUE	6 hours	12 hour	1 day	1.5 days	2 days	3 days	7 days
Trichlorfon							
Muscle close to dosing site	-	<50	90 <50 (3)	-	-	<50	<50
Fat close to dosing site	-	173, 70, 51, 142	2350 <50 (3)	-	-	<50	<50
Milk	79±57	61±84	28 <25 (7)	29 <25 (7)	<25	<25	-
Dichlorvos							
Muscle close to dosing site	-	<50	<50	-	-	<50	<50
Fat close to dosing site	-	<50	142, <50 (3)	-	-	<50	<50
Milk	<25 (3) <2.5 (5)	<25 (1) <2.5 (7)	<2.5	<2.5	<2.5	<2.5	-

Horse

Three horses were administered a single oral dose of 35 mg trichlorfon /kg BW in a paste combination with 6 mg febental/kg BW. The horses were sacrificed 14 days later and samples of fat and muscle assayed for the sum of trichlorfon and dichlorvos measured as the common breakdown product, dimethyl phosphite, by gas chromatography. All residues were < LOD, 50 μg/kg (Dorn and Blass, 1982).

BOUND RESIDUES AND BIOAVAILABILITY

The majority of the tissue residues of [ethyl-^{14}C]-trichlorfon applied topically to cattle was extractable (see table 3). Following a more rigorous pepsin/HCl digest and extraction procedure for muscle, liver and kidney tissues (after the initial mild solvent extraction), the additional amount of total residues was 23 and 25% in muscle, 15 and 19% in liver and 5 and 17% in kidney (Phillips, 1998). The milder extraction procedure for fat resulted in 14-30% of the residues being non-extractable. The nature of the bound residues is unknown.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A validated GC-MS method for the identification and quantification of trichlorfon residues in the liver, kidney, muscle and fat of cattle, using d_6-trichlorfon as an internal standard, has been reported (Krebber, 1998). A modification of this method, using an external standard added to the sample matrix blank that has been taken through the isolation procedure has been validated for the analysis of trichlorfon residues in tissues and milk of cattle (Krebber, 1999a) and in the liver, kidney, muscle and fat of horse (Krebber, 1999b).

Trichlorfon residues are extracted from either tissues or milk with acetonitrile containing 0.1% formic acid. For liver, kidney and muscle, the combined extracts were diluted with water, extracted with dichloromethane, evaporated to dryness and reconstituted with n-heptane. For milk and fat, the acetonitrile extract was partitioned with n-heptane, the acetonitrile phase evaporated to dryness and reconstituted with n-heptane.

For sample clean-up, n-heptane extracts were added to a silica cartridge, washed with ethyl acetate-heptane (1:9) and trichlorfon eluted with ethyl acetate. For cattle tissue samples, the ethyl acetate elutate was evaporated, reconstituted with a small volume of ethyl acetate and the internal standard (d_6-Trichlorfon) added. For analyses where external the external standard was used, an extract derived by subjecting residue-free tissue or milk to the entire extraction procedure was fortified with trichlorfon immediately prior to GC-MS analysis. Trichlorfon was detected by selected ion monitoring at m/z 109 with selective ion monitoring at m/z 115 for d_6-trichlorfon.

Performance characteristics of the method

The method was validated for use over the linear range of detector response, 0.01-1 mg/kg for tissues and 0.05-2 mg/kg for milk. Detection in the selective ion monitoring mode, together with an established GC retention time ensure that the method has a high degree of specificity. Other performance characteristics of the method, using either internal or external standard are shown in Table 5.

Table 5. Recovery data for internal and external standard variations of the GC-MS trichlorfon residue method at different spiking concentrations.

Matrix	% Recovery (%SD)								
	Cattle-internal standard			Cattle-external standard			Horse-external standard		
	L*	M	H	L*	M	H	L*	M	H
Liver	76 (7.3)	83 (6.3)	84 (6.8)	81 (5.7)	81 (5.6)	85 (5.4)	81 (7)	70 (17)	73 (5)
Kidney	90 (2.4)	78 (8.4)	72 (8.8)	83 (7.6)	86 (7.8)	86 (10.4)	76 (15)	80 (11)	98 (6)
Muscle	88 (3.2)	86 (2.7)	83 (3.7)	96 (7.8)	88(14.9)	82 (9.9)	77 (14)	71 (10)	72 (19)
Fat	87 (13.0)	101 (4.2)	101 (4.0)	96(20.8)	112 (17.1)	97 (8.1)	78 (21)	101 (11)	87 (11)
Milk				75(13)	93 (12)	94 (18)			

Note. * = LOQ values are 0.05 mg/kg for tissue and 0.025 mg/kg for milk. L = 0.05 mg/kg for tissue (0.025 mg/kg for milk); M = 0.1 mg/kg (0.05 mg/L for milk); H = 0.2 mg/kg (0.1 mg/L for milk)

The limit of detection (LOD) of the method in cattle was 9 µg/kg for liver and fat, 6 µg/kg for kidney and fat and 2 µg/kg in milk. In the horse, using an external standard, the LOD was 20 µg/kg in liver, 16 µg/kg in kidney, 8 µg/kg in muscle and 9 µg/kg in fat. The limit of quantification (LOQ) was 50 µg/kg in all tissues and 25 µg/kg in milk. The recoveries obtained from each tissue and milk at the LOQ are shown in table 5.

This method proposed by the sponsor for routine surveillance purposes has no reported matrix interference. The non-commercial availability of the internal standard would require that the external standard method should be used or the introduction of a suitable surrogate standard investigated.

The possibility of determination of dichlorvos by this method has not been reported. It is possible that, give the chemical and physical properties of trichlorfon, that diclorvos residues could be detected and quantified as part of a multi-residue organophosphate (OP) screen used for OP residues by regulatory agencies.

APPRAISAL

Both oral and topical administration of trichlorfon is indicated for cattle, only an oral preparation is available for the horse. The new data for topical administration was developed in compliance with GLP. None of the older data for the oral administration is GLP compliant and is only of limited coverage.

The metabolic pathways for the rat and rabbit appear more substantiated than for ruminants. The biotransformations in the cow, goat or the horse have not been clearly established. It proved difficult to quantify the metabolites in the cattle administered [ethyl-14C]-trichlorfon (see table 2). Nevertheless, in all species there is evidence of rapid metabolism following absorption. Trichlorfon and dichlorvos account for very low percentages of the total residues in the major edible tissues and milk of cattle.

Both trichlorfon and dichlorvos have insecticidal activity and more information on the insecticidal properties of the other metabolites would have been valuable. It is not known whether the other metabolites are also toxic.

The persistence of the active compound at the site of pour-on application is necessary for long ecto-parasiticidal action. This persistence alters the pharmacokinetics of trichlorfon as a pour-on treatment compared with the oral administration. The levels of radioactivity in plasma and tissues distant from the pour-on dose area appear to peak at 3 days and are depleting by five days post-dosing (see table 3 and figure 2). The persistence of residues may be due to the continuing absorption of the large part of the dose remaining on the skin. Studies beyond a 5 day post-dosing period would have provided better information on the rate of depletion of the total residues. There was considerable variability between cattle in the amount of run-off dose, 14–49%. If similar large differences were observed under field conditions it could affect both efficacy and withdrawal times for individual cattle.

Numerous residue depletion studies using unlabelled drug administered either orally or topically were performed during 1959-69 (before GLP). The analytical methods were not generally specific for trichlorfon or dichlorvos. The sponsors have submitted chromatographic studies but not those using other end-point measurements such as the determination of residues using the inhibition of cholinesterase enzyme activity. It may possible to correlate the inhibition of cholinesterase activity with later results in which it is certain that no residues of trichlorfon and dichlorvos are found. Two new GLP compliant studies were submitted by the sponsors in which the residues of trichlorfon and dichlorvos were measured in tissues and milk of cattle after topical application of unlabelled trichlorfon. At intervals during a 7 day observation period, no residues of trichlorfon were detected (LOD = 50 µg/kg) in muscle and fat distant from the dosing area or in liver and kidney. No residues (LOD = 50 µg/kg) of dichlorvos were found in any tissue except in fat tissue close to the dose site taken from one animal at day 1. Samples taken from this animal of muscle and fat close to the site of application also contained residues of trichlorfon. The results for muscle and fat close to the dosing area are shown in table 4. Residues were found in the subcutaneous fat close to the dose area in all four animals. 12 hours after dosing. In the second study dairy cows were treated with a single spray of 5% solution of trichlorfon at a dose rate of 40 mg trichlorfon/kg B.W. Trichlorfon was excreted in the milk, mostly during the first 12 hours post-dosing. The highest level was 205 µg/kg in one cow sampled at 6 h post-dose. Residues were present just above the LOQ (25 µg/kg) in one cow at 24 hours and another cow at 36 hours but thereafter no residues were detected (LOD 2.5 µg/kg).

A validated GC-MS method for the identification and quantification of trichlorfon residues in the liver, kidney, muscle and fat of cattle, which uses d6-Trichlorfon as an internal standard, has been reported. A modification of this method using an external standard added to the sample matrix blank has been taken through the isolation procedure. It has been validated for the analysis of trichlorfon residues in tissues and milk of cattle (Krebber, 1999a) and in the liver, kidney, muscle and fat of horse (Krebber, 1999b). No interference from the matrix has been reported in this method, which is proposed by the sponsor for use in routine surveillance. Since the internal standard is not available commercially, either

the method involving external standard should be used or the introduction of a suitable surrogate standard should be investigated.

MAXIMUM RESIDUE LIMITS

The ADI is 0–0.02 mg/kg of body weight, equivalent to a maximum of 1200 μg per 60-kg person. In reaching its decision, the Committee considered the following:

1. Only trichlorfon and dichlorvos are of toxicological concern. Trichlorfon is a pro-drug, and dichlorvos is the only metabolite with effective insecticidal action.
2. Dichlorvos is very unstable and is not found in animal tissues or milk.
3. The metabolism of trichlorfon in target and laboratory animals is broadly similar.
4. Trichlorfon is metabolized so extensively and rapidly that the ratio of marker residue to total residues cannot be defined.
5. There is a suitable routine analytical method for determining trichlorfon, with an LOQ of 50 μg/kg for muscle, liver, kidney, and fat and 25 μg/kg for milk.
6. The concentrations of trichlorfon in tissues distant from the site of application were below the LOQ.
7. Within one day of administration of a pour-on preparation, the concentrations of residues in muscle and fat samples collected close to the site of application were above the LOQ in a few animals. By 3 days after administration, no residues were present in fat or muscle close to the site of administration.
8. Residues of trichlorfon were found at concentrations above the LOQ from the first three milkings after treatment, but thereafter the concentrations were below the LOQ. No residues of dichlorvos were found at concentrations greater than the LOQ (25 μg/kg) in any sample of milk.
9. The TMDI for total residues calculated for muscle, liver, kidney and fat in the pivotal radiodepletion study in calves was 150 μg, representing only 12.5% of the ADI.
10. Insufficient information was available to extend the MRLs to horses.

The MRLs for muscle, liver, kidney and fat should serve as guidelines only for the control of residues. Residues of trichlorfon were not detected in the residue depletion studies reviewed by the Committee. The Committee believes that residues in muscle, liver, kidney and fat would not be found at the limit of quantification of available analytical methods. MRLs were not allocated by the Committee for muscle, liver, kidney and fat considering that no detectable residues should be present in tissues from animals treated with trichlorfon when used in accordance with good practice in the use of veterinary drugs. Therefore, the LOQ may be used as guideline maximum residue concentrations in muscle, liver, kidney and fat by National governments. The guidance values are 50 μg/kg for muscle, liver, kidney and fat in cattle and an MRL of 50 μg/kg for milk measured as parent drug. The TMDI is 75 μg for milk.

The Committee requests the development of an analytical method for trichlorfon with LOQs at least one-half of the current values.

REFERENCES

Ahr, H.J. and Siefert, H.M. (1992). [^{14}C]BAYa9826 (Trichlorfon). Absorption, plasma concentrations, Excretion and enterohepatic circulation of total radioactivity following single i.v., p.o. or i.d. administration to male rats. Bayer Report No. 21401.

Ahr, H.J. and Zimmer, D. (1994). Pharmacokinetics of the enantiomers and DDVP (BAYb4986) in male rabbits after single oral administration of Trichlorfon (67 mg/kg) and single i.v. administration of DDVP (1mg/kg) . [I300562]. Bayer Report No. 23384

Ahr, H.J., Boberg, M. and Siefert, H.M. (1993). [^{14}C]BAYa9826 (Trichlorfon). Pharmacokinetics and metabolism in male rabbits following single oral administration.

Aur, S. and Krebber, R. (1998). Determination of residues of trichlorfon and dichlorvos in edible tissues (muscle, fat, kidney and liver) 12 hours to 7 days after a single spray treatment on cattle. Bayer Report No. V97-002.

Aur, S Gvozdenovic, S. and Krebber, R. (1999). Neguvon Powder ® - Residue study in dairy cows. Bayer Report No. V98-003

Boberg, M., Ahr. H.J., Kanhai, W. and Karl, W. (1993). [^{14}C]BAYa9826 (Trichlorfon). Biotransformation in rats. Bayer Report No. 22804.

Boberg, M., Kanhai, W. and Karl, W. (1994). [^{14}C]BAYa9826 (Trichlorfon). Biotransformation inRhesus monkeys. Bayer Report No. 23313.

Cornelissen, K. (1993). [^{14}C]BAYa9826 (Trichlorfon). Absortion, metabolism and excretion following oral and intravenous administration to the Rhesus monkey. Bayer Report No. 6056.

Dorn, H. and Blass, W. (1982). Determination of Neguvon and dichlorvos after simultaneous treatment of horses with Rintal and Neguvon paste. Supplement to Bayer Document, 82/10581.

Krebber, R. (1998). Method for the GC/MS determination of trichlorfon in cattle tissue. Report No. MR-952/97, ID 18418 (Routine analytical method)

Krebber, R. (1999a). Method for the GC/MS determination of trichlorfon in Milk. Report No. MR-820/98, ID 18418 (Routine analytical method)

Krebber, R. (1999b). Method for the GC/MS determination of trichlorfon in Horse Tissue. Report No. MR-920/98, ID 18418 (Routine analytical method)

Lynch, C.J. and Speirs, G.C. (1998). [ethyl-^{14}C]-trichlorfon: Total Residue depletion study in cattle after dermal application (in-life phase). Inveresk Report No, 15124.

Phillips. M. (1998). [ethyl-^{14}C]-trichlorfon: Investigation of the nature of metabolites in edible tissues of cattle after dermal application (animals 3M to 8M).

Phillips, M. and Johnson, S. (1998). [ethyl-^{14}C]-trichlorfon: Investigation of the nature of metabolites in edible tissues of cattle after dermal application (animals 1F and 2M).

Robbins, W.E., Hopkins, T.H. and Eddy, G.W. (1956). The metabolism of P^{32}-labeled L13/59 in a cow. J. Econ. Entomol. 49, 801-806

Schwarz, T., Ahr, H.J., Goller, G. and Steinke, W. (1994). [^{14}C]BAYa9826 (Trichlorfon). Distribution of radioactivity and elimination from plasma, organs, and tissues of male albino rats after single and repeated oral administration. Bayer Report No. 23410.

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

This attached table summarises the veterinary drug evaluations conducted by JECFA at the 32nd (1987), 34th (1989), 36th (1990), 38th (1991), 40th (1992), 42nd (1994), 43rd (1994), 45th (1995), 47th (1996), 48th (1997), 50th (1998), 52nd (1999) and 54th (2000) meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in food. Please see reports of those meetings, published in WHO Technical Report Series (TRS).

Some notes regarding the Table:

- The "Status" column refers to the ADI and indicates if "No" ADI was established, if a full ADI was given, or if the ADI is Temporary (T).
- Where an MRL is temporary, it is so indicated by "T".
- Several compounds have been evaluated more than once. The data given are for the most recent evaluation, including the 54th meeting of the Committee.

Substance	ADI (µg/kg bw)	ADI Status	JECFA	MRL (µg/kg)	Tissue	Species	Marker residue and other remarks
Abamectin	0-1 (1995 JMPR)	Full	47 (1996)	100 50	Liver, fat Kidney	Cattle	Avermectin B_{1a}
Albendazole	0-50	Full	34 (1989)	100 5000	Muscle, fat, milk Liver, kidney	Cattle, sheep	MRLs analysed as 2-amino-benzimidazole and expressed as parent drug equivalents, see WHO TRS 788
Azaperone	0-6	Full	50 (1998)	60 100	Muscle, fat Liver, kidney	Pigs	Sum of azaperone and azaperol
Benzylpenicillin	30 µg/person/day	Full	36 (1990)	50 4	Muscle, liver, kidney Milk	All species	Parent drug
Bovine Somatotropins	Not specified	Full	50 (1998)	Not specified	Muscle, liver, kidney, fat, milk	Cattle	
Carazolol	0-0.1	Full	43 (1994)	5 25	Muscle, fat/skin Liver, kidney	Pigs	Parent drug. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI which is based on the acute pharmacological effect of carazolol
Carbadox	Limited acceptance	Full	36 (1990)	30 5	Liver Muscle	Pigs	Quinoxaline-2-carboxylic acid

Ceftiofur	0-50	Full	45 (1995) 48 (1997)	 1000 2000 6000 2000 100 µg/l	 Muscle Liver Kidney Fat Milk	 Cattle, pigs Cattle	Desfuroylceftiofur
Chloramphenicol	No ADI		42 (1994)	No MRL			
Chlorpromazine	No ADI		38 (1991)	No MRL			
Chlortetracycline, oxytetracycline, tetracycline	0-30 (Group ADI)	Full	50 (1998) 54 (2000)	200 600 1200 400 100 µg/l 100 200 T	Muscle Liver Kidney Eggs Milk Muscle Muscle	Cattle, pigs, sheep, poultry Poultry Cattle, sheep Giant prawn Fish	Parent drugs, singly or in combination Oxytetracycline only Oxytetracycline only
Clenbuterol	0-0.004	Full	47 (1996)	0.2 0.6 0.05 µg/l	Muscle, fat Liver, kidney Milk	Cattle, horses Cattle	Parent drug
Closantel	0-30	Full	36 (1990) 40 (1992)	 1000 3000 1500 5000 2000	 Muscle, liver Kidney, fat Muscle, liver Kidney Fat	 Cattle Sheep	Parent drug
Cyfluthrin	0-20	Full	48 (1997)	20 200 40 µg/l	Muscle, liver, kidney Fat Milk	Cattle	Parent drug
Cyhalothrin	0-2	T	54 (2000)	20 T 400 T 30 µg/kg T	Muscle, liver, kidney Fat Milk	Cattle, pig, sheep Cattle	Parent drug
Cypermethrin	0-50	Full	47 (1996)				Parent drug
α-Cypermethrin	0-20	Full	47 (1996)				

Danofloxacin	0-20	Full	48 (1997)	200 400 100 100 50 200 100	Muscle Liver, kidney Fat Muscle Liver Kidney Fat	Cattle, chickens Pigs	Parent drug For chickens fat/skin in normal proportion
Deltamethrin	0– 0 (JMPR 1982)	Full	52 (1999)	30 (guidance) 50 500 30 30	Muscle Liver, kidney Fat Milk Egg	Cattle, sheep, chicken, salmon Cattle, sheep, chicken Cattle, sheep, chicken Cattle Chicken	Parent drug
Dexamethasone	0-0.015	Full	42 (1994) 48 (1997) 50 (1998)	 No MRL No MRL			 Temporary MRLs were not extended Regulatory method not available
Diclazuril	0-30	Full	50 (1998)	500 3000 2000 1000	Muscle Liver Kidney Fat	Sheep, rabbits, poultry	Parent drug Poultry skin/fat
Dicyclanil	0-7	Full	54 (2000)	200 400 150	Muscle Liver, kidney Fat	Sheep	Parent drug
Dihydrostrepto-mycin, streptomycin	0-50 (Group ADI)	Full	48 (1997) 52 (1999)	 600 1000 200 µg/kg T	 Muscle, liver, fat Kidney Milk	 Cattle, pigs, sheep, chickens Cattle	Sum of dihydrostreptomycin and streptomycin
Demetridazole	No ADI		34 (1989)	No MRL			
Diminazene	0-100	Full	42 (1994)	500 12000 6000 150 µg/l	Muscle Liver Kidney Milk	Cattle	Parent drug
Doramectin	0-0.5	Full	45 (1995) 52 (1999)	10 5 100 30 150	Muscle Muscle Liver Kidney Fat	Cattle Pigs Cattle, Pigs	Parent drug. The Committee noted the high concentration of residues at the injection site over a 35-day period after subcutaneous or intramuscular administration of the drug at the recommended dose.
Enrofloxacin	0-2	Full	48 (1997)	No MRL			

Eprinomectin	0-10	Full	50 (1998)	100 2000 300 250 20 µg./l	Muscle Liver Kidney Fat Milk	Cattle	Eprinomectin B_{1a}
Estradiol-17β	0-0.05	Full	52 (1999)	Not specified	Muscle, liver, kidney, fat	Cattle	
Febantel, fenbendazole, oxfendazole	0-7 (Group ADI)	Full	50 (1998)	100 500 100 µg/l	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs, horses, goats Cattle, sheep	Sum of fenbendazole, oxfendazole, and oxfendazole sulfone, expressed as oxfendazole sulfone equivalents
Fenbendazole (see febantel)							
Fluazuron	0-40	Full	48 (1997)	200 500 7000	Muscle Liver, kidney Fat	Cattle	Parent drug
Flubendazole	0-12	Full	40 (1992)	10 200 500 400	Muscle, liver Muscle Liver Eggs	Pigs Poultry	Parent drug
Flumequine	0-30	Full	48 (1997) 54 (2000)	500 3000 1000 500 500 500 3000 1000	Muscle Kidney Fat Liver Muscle Muscle, liver Kidney Fat	Cattle Cattle Trout Pigs, sheep, chickens	Parent drug Muscle/skin in normal proportion
Furazolidone	No ADI		40 (1992)	No MRL			
Gentamicin	0-20	Full	50 (1998)	100T 2000 5000 200 µg/l	Muscle, fat Liver Kidney Milk	Cattle, pigs Cattle	Parent drug
Imidocarb	0-10	Full	50 (1998)	300 T 2000 T 1500 T 50 T 50 µg/l T	Muscle Liver Kidney Fat Milk	Cattle	Parent drug
Ipronidazole	No ADI		34 (1989)	No MRL			

Isometamidium	0-100	Full	40 (1992)	100 500 1000	Muscle, fat, milk Liver Kidney	Cattle	Parent drug
Ivermectin	0-1	Full	40 (1992) 54 (2000)	100 40 15 20 10 T	Liver Fat Liver Fat Milk	Cattle Pigs, sheep Cattle	Ivermectin H_2B_{1a}
Levamisole	0-6	Full	42 (1994)	10 100	Muscle. Kidney, fat Liver	Cattle, sheep, pigs, poultry	Parent drug
Lincomycin	0-30	Full	54 (2000)	100 500 1500 150 100 T 500 T 1500 T	Muscle, fat Liver Kidney Milk Muscle, fat Liver Kidney	Pigs Cattle Cattle, sheep, chicken	Parent drug
Melengestrol acetate	0-0.03	Full	54 (2000)	2 T 5 T	Liver Fat	Cattle	Parent drug MRL temporary as no practical analytical method for residue control.. No MRL recommended for muscle because of very low residues and inadequate analytical method.
Metronidazole	No ADI		34 (1989)	No MRL			
Moxidectin	0-2	Full	45 (1995) 47 (1996) 48 (1997) 50 (1998)	100 50 500 50 20 20 100 50 500	Liver Kidney Fat Muscle Muscle Muscle Liver Kidney Fat	Cattle, sheep Sheep Cattle Deer	Parent drug. The Committee noted the very high concentration and great variation in the level of residues at the injection site in cattle over a 49-day period after dosing.

Neomycin	0-60	Full	47 (1996) 52 (1999) 52 (1999) 500 500 µg/kg	500 15000 500 20000 10000 500 500 µg/kg	Muscle, fat Liver Liver Kidney Kidney Eggs Milk	Cattle, chicken, duck, goat, pig, sheep, turkey Cattle Chicken, duck, goat, pig, sheep, turkey Cattle Chicken, duck, goat, pig, sheep, turkey Chicken Cattle	Parent drug
Nicarbazin	0-400	Full	50 (1998)	200	Muscle, liver, kidney, fat/skin	Chicken (broilers)	
Nitrofurazone	No ADI		40 (1992)	No MRL			
Olaquindox	Limited acceptance	T	42 (1994)	No MRL (see remarks)	Muscle	Pigs	MQCA. The Committee recommended no MRLs but noted that 4 µg/kg of MQCA (T) is consistent with Good Veterinary Practice
Oxfendazole (see febantel)							
Oxolinic acid	No ADI		43 (1994)	No MRL			
Oxytetracycline (see chlortetracycline)							
Phoxim	0-4	Full	52 (1999)	50 T 400 T 10 T	Muscle, liver, kidney Fat Milk	Cattle, pig, sheep, goat Cattle, pig, sheep, goat Cattle	Parent drug
Porcine somatotropins	Not specified		52 (1999)	Not specified	Muscle, liver, kidney, fat	Pigs	
Procaine benzylpenicillin	Less than 30 µg of penicillin per person per day	Full	50 (1998)	50 4 µg/kg	Muscle, liver, kidney Milk	Cattle, pigs, chickens Cattle	Benzylpenicillin
Progesterone	0-30	Full	52 (1999)	Not specified	Muscle, liver, kidney, fat	Cattle	
Propionyl-promazine	No ADI		38 (1991)	No MRL			
Ractopamine	No ADI		40 (1992)	No MRL			

Ronidazole	Withdrawn		42 (1994)	No MRL			
Sarafloxacin	0-0.3	Full	50 (1998)	10 80 20	Muscle Liver, kidney Fat	Chicken, turkey Chicken, turkey Chicken, turkey	Parent drug
Spectinomycin	0-40	Full	42 (1994) 50 (1998)	 500 2000 5000 2000 200 µg/kg	 Muscle Liver, fat Kidney Eggs Milk	Cattle, pig, sheep, chicken Chicken Cattle	Parent drug
Spiramycin	0-50	Full	43 (1994) 47 (1996) 48 (1997)	 200 600 300 800 300 200 µg/kg	 Muscle Liver Kidney Kidney Fat Milk	 Cattle, chicken, pig Cattle, chicken, pig Cattle, pig Chicken Cattle, chicken, pig Cattle	For cattle and chickens MRLs are expressed as the sum of spiramycin and neospiramycin For pigs MRLs expressed as spiramycin equivalents (antimicrobially active residues)
Streptomycin (see dihydrostrepto-mycin							
Sulfadimidine	0-50	Full	42 (1994)	100 25 µg/kg	Muscle, liver, kidney, fat Milk	Cattle, sheep, pig, poultry Cattle	Parent drug
Sulphthiazole	No ADI		34 (1989)	No MRL			
Testosterone	0-2	Full	52 (1952)	Not specified	Muscle, liver, kidney, fat	Cattle	
Tetracycline (see Chlortetracycline)							
Thiamphenicol	0-5	Full	52(1999)	50 T 100 T 500 T 50 T	Muscle, fat Liver Kidney Muscle	Pigs Pigs Pigs Fish	Thiamphenicol and thiamphenicol conjugates measured as thiamphenicol
Thiabendazole	0-100	Full	48 (1997) 40 (1992)	 100 100 µg/kg	 Muscle, liver, kidney, fat Milk	 Cattle, pig, goat, sheep Cattle, goat	Sum of thiabendazole and 5-hydroxythiabendazole

Tilmicosin	0-40	Full	47 (1996)	100 1000 1500 300 1000 50 µg/kg T	Muscle, fat Liver Liver Kidney Kidney Milk	Cattle, pig, sheep Cattle, sheep Pig Cattle, sheep Pig' Sheep	Parent drug
Trenbolone acetate	0-0.02	Full	34 (1989)	2 10	Muscle Liver	Cattle	β-Trenbolone for muscle α-trenbolone for liver
Trichlorfon (Metrifonate)	0-20	Full	54 (2000)	50 µg/kg 50	Milk Muscle, liver, kidney, fat	Cattle Cattle	Parent drug Guidance MRLs (No residues detected in depletion studies. No residues should be present in tissues when used with good veterinary practice. .Limit of quantification used as guideline MRL)
Triclabendazole	0-3	Full	40 (1992)	200 300 100 100	Muscle Liver, kidney Fat Muscle, liver kidney, fat	Cattle Sheep	5-Chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one
Tylosin	No ADI		38 (1991)	No MRL			
Xylazine	No ADI		47 (1996)	No MRL			
Zeranol	0-0.5	Full	32 (1987)	2 10	Muscle Liver	Cattle	Parent drug

ANNEX 2

SUMMARY OF 54th JECFA RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

Anthelminthic agent

Ivermectin

Acceptable daily intake: 0 –1 µg/kg bw (established at the fortieth meeting of the Committee (WHO TRS[1] 832, 1993))

Residue definition: Ivermectin H_2B_{1a}

Recommended maximum residue limits (MRLs)[a]

Species	Milk (µg/kg)
Cattle	10[b]

[a]The MRLs that were recommended at the thirty-sixth and fortieth meetings of the Committee (WHO TRS 799 (1990) and 832 (1993), respectively) were not reconsidered and were maintained.
[b]Temporary MRL. Validation data on the analytical method and information on other routes of application to cattle to evaluate the residues in milk are required for evaluation in 2002.

Antimicrobial agents

Flumequine

Acceptable daily intake: 0 – 30 µg/kg bw (established at the forty-eighth meeting of the Committee (WHO TRS 879, 1998))

Residue definition: Flumequine

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Cattle	500	500	3000	1000
Pigs	500	500	3000	1000
Sheep	500	500	3000	1000
Chickens	500	500	3000	1000
Trout	500[a]			

[a] Trout muscle including skin in natural proportions.

[1] TRS: Technical Report Series

Lincomycin

Acceptable daily intake: 0 – 30 µg/kg bw
Residue definition: Lincomycin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/kg)
Cattle	100[a]	500[a]	1500[a]	100[a]	150
Pigs	100	500	1500	100	
Sheep	100[a]	500[a]	1500[a]	100[a]	
Chickens[b]	100[a]	500[a]	1500[a]	100[a]	

[a]The recommended MRLs are temporary. Data comparable to those provided for tissues of pigs, which show that lincomycin is the major component with significant microbiological activity in tissues of cattle, sheep, and chickens, are required for evaluation in 2002.
[b]Before considering an MRL in chicken eggs, the Committee would wish to see (1) data showing that lincomycin is the major component with significant microbiological activity and (2) a residue depletion study using the GC/MS method.

Oxytetracycline

Acceptable daily intake: 0 – 30 µg/kg bw (group ADI for tetracycline, oxytetracycline and chlortetracycline; established at the fiftieth meeting of the Committee (WHO TRS 888, 1999))
Residue definition: Oxytetracycline, singly or in combination with chlortetracycline and tetracycline.

Recommended maximum residue limits (MRLs)[a]

Species	Muscle (µg/kg)
Fish	200[b]

[a]The MRLs that were recommended in cattle, pigs, sheep, poultry, and giant tiger prawn (*Penaeus monodon*) at the fiftieth meeting of the Committee (WHO TRS 888, 1999) were not reconsidered and were maintained.
[b]Temporary MRL. Residue data and a validated analytical method are required for evaluation in 2002.

Tilmicosin

Acceptable daily intake: 0 – 40 µg/kg body weight (established at the forty-seventh meeting of the Committee (WHO TRS 876, 1996))
Residue definition: Tilmicosin

Recommended maximum residue limits (MRLs)[a]

Species	Milk (µg/kg)
Sheep	not extended[b]

[a]The MRLs that were recommended in muscle, liver, kidney, and fat of cattle, pigs, and sheep at the forty-seventh meeting of the Committee (WHO TRS 876, 1998) were not reconsidered and were maintained.
[b] The temporary MRL for sheep milk was not extended as results of a study with radiolabelled drug in lactating sheep to determine the relationship between total residues and parent drug in milk were not available.

Insecticides

Cyhalothrin

Acceptable daily intake: 0 – 2 µg/kg bw (temporary)[a]
Residue definition: Cyhalothrin

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/kg)
Cattle[b]	20	20	20	400	30
Pigs[b]	20	20	20	400	
Sheep[b]	20	20	20	400	

[a]Results of appropriate studies to establish a no-observed-effect level (NOEL) for neurobehavioral effects in laboratory animals are required for evaluation in 2002.
[b]The recommended MRLs are temporary because the ADI is temporary. In addition, results of the analytical method validation for sheep liver, to demonstrate a limit of quantification of 0.01 mg/kg, are required for evaluation in 2002.

Cypermethrin

Acceptable daily intake: 0 – 50 µg/kg bw (established at the forty-seventh meeting of the Committee (WHO TRS 876, 1998))
Residue definition: Cypermethrin, sum of isomers

Recommended maximum residue limits (MRLs)

Species	Muscle	Liver	Kidney	Fat	Milk	Eggs
Cattle[a]	Not extended	Not extended	Not extended	Not extended	Not extended	
Sheep[a]	Not extended	Not extended	Not extended	Not extended		
Chickens[a]	Not extended	Not extended	Not extended	Not extended		Not extended

[a]Since the information required at the forty-seventh meeting of the Committee (WHO TRS 876, 1998) was not provided and there was no indication that it would be provided in the future the temporary MRLs that had been recommended for cattle, sheep and chickens were not extended.

***alpha*-Cypermethrin**

Acceptable daily intake: 0 – 20 µg/kg bw (established at the forty-seventh meeting of the Committee (WHO TRS 876, 1998))
Residue definition: *alpha*-Cypermethrin, sum of isomers

Recommended maximum residue limits (MRLs)

Species	Muscle	Liver	Kidney	Fat	Milk	Eggs
Cattle[a]	Not extended	not extended	not extended	not extended	not extended	
Sheep[a]	Not extended	not extended	not extended	not extended		
Chickens[a]	Not extended	not extended	not extended	not extended		not extended

[a]Since the information required at the forty-seventh meeting of the Committee (WHO TRS 876, 1998) was not provided and there was no indication that it would be provided in the future the temporary MRLs that had been recommended for cattle, sheep and chickens were not extended.

Dicyclanil

Acceptable daily intake: 0 – 7 µg/kg bw
Residue definition: Dicyclanil

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)
Sheep	200	400	400	150

Permethrin

Acceptable daily intake: 0 – 50 µg/kg bw (for technical grade permethrin with *cis:trans* ratios of 25:75 to 40:60; established by the 1999 JMPR (FAO Plant Production and Protection Paper, in press; available at http://www.fao.org/ag/agp/ agpp/pesticid/ or http://www.who.int/pcs/jmpr/jmpr.htm))
Residue definition: Permethrin, sum of isomers

Recommended maximum residue limits (MRLs)

The Committee concluded that the database available to the 1999 Joint FAO/WHO Meeting on Pesticide Residues (JMPR) was not adequate to assess the toxicity of the 80:20 *cis:trans* isomeric mixture proposed for use as a veterinary drug. In absence of an ADI the Committee was unable to establish MRLs for this isomeric mixture of permethrin.

Trichlorfon (metrifonate)

Acceptable daily intake: 0 – 20 µg/kg bw
Residue definition: Trichlorfon

Recommended maximum residue limits (MRLs)

Species	Muscle (µg/kg)	Liver (µg/kg)	Kidney (µg/kg)	Fat (µg/kg)	Milk (µg/kg)
Cattle	50[a]	50[a]	50[a]	50[a]	50

[a] Guidance value only. Residues of trichlorfon (metrifonate) were not detected in the residue depletion studies reviewed by the Committee. MRLs were not recommended by the Committee for muscle, liver, kidney or fat in cattle considering that no detectable residues should be present in tissues from animals treated with trichlorfon when used in accordance with good practice in the use of veterinary drugs. The limit of quantification may be used as guideline maximum residue concentrations in muscle, liver, kidney and fat of cattle.

Production aid

Melengestrol acetate

Acceptable daily intake: 0 – 0.03 µg/kg bw
Residue definition: Melengestrol acetate

Maximum residue limits (MRLs)

Species	Liver (µg/kg)	Fat (µg/kg)
Cattle	2[a]	5[a]

[a]Temporary MRL. A practical analytical method for monitoring residues of melengestrol acetate at the recommended MRL is required for evaluation in 2002.

FAO TECHNICAL PAPERS

FAO FOOD AND NUTRITION PAPERS

1/1	Review of food consumption surveys 1977 – Vol. 1. Europe, North America, Oceania, 1977 (E)
1/2	Review of food consumption surveys 1977 – Vol. 2. Africa, Latin America, Near East, Far East, 1979 (E)
2	Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E F S)
3	Report of a joint FAO/WHO expert consultation on dietary fats and oils in human nutrition, 1977 (E F S)
4	JECFA specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers, 1978 (E)
5	JECFA – guide to specifications, 1978 (E F)
5 Rev. 1	JECFA – guide to specifications, 1983 (E F)
5 Rev. 2	JECFA – guide to specifications, 1991 (E)
6	The feeding of workers in developing countries, 1976 (E S)
7	JECFA specifications for identity and purity of food colours, enzyme preparations and other food additives, 1978 (E F)
8	Women in food production, food handling and nutrition, 1979 (E F S)
9	Arsenic and tin in foods: reviews of commonly used methods of analysis, 1979 (E)
10	Prevention of mycotoxins, 1979 (E F S)
11	The economic value of breast-feeding, 1979 (E F)
12	JECFA specifications for identity and purity of food colours, flavouring agents and other food additives, 1979 (E F)
13	Perspective on mycotoxins, 1979 (E F S)
14	*Manuals of food quality control:*
14/1	Food control laboratory, 1979 (Ar E)
14/1 Rev.1	The food control laboratory, 1986 (E)
14/2	Additives, contaminants, techniques, 1980 (E)
14/3	Commodities, 1979 (E)
14/4	Microbiological analysis, 1979 (E F S)
14/5	Food inspection, 1981 (Ar E) (Rev. 1984, E S)
14/6	Food for export, 1979 (E S)
14/6 Rev.1	Food for export, 1990 (E S)
14/7	Food analysis: general techniques, additives, contaminants and composition, 1986 (C E)
14/8	Food analysis: quality, adulteration and tests of identity, 1986 (E)
14/9	Introduction to food sampling, 1988 (Ar C E F S)
14/10	Training in mycotoxins analysis, 1990 (E S)
14/11	Management of food control programmes, 1991 (E)
14/12	Quality assurance in the food control microbiological laboratory, 1992 (E F S)
14/13	Pesticide residue analysis in the food control laboratory, 1993 (E F)
14/14	Quality assurance in the food control chemical laboratory, 1993 (E)
14/15	Imported food inspection, 1993 (E F)
14/16	Radionuclides in food, 1994 (E)
14/17	Unacceptable visible can defects – a pictorial manual, 1998 (E F S)
15	Carbohydrates in human nutrition, 1980 (E F S)
16	Analysis of food consumption survey data for developing countries, 1980 (E F S)
17	JECFA specifications for identity and purity of sweetening agents, emulsifying agents, flavouring agents and other food additives, 1980 (E F)
18	Bibliography of food consumption surveys, 1981 (E)
18 Rev. 1	Bibliography of food consumption surveys, 1984 (E)
18 Rev. 2	Bibliography of food consumption surveys, 1987 (E)
18 Rev. 3	Bibliography of food consumption surveys, 1990 (E)
19	JECFA specifications for identity and purity of carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents and other food additives, 1981 (E F)
20	Legumes in human nutrition, 1982 (E F S)
21	Mycotoxin surveillance – a guideline, 1982 (E)
22	Guidelines for agricultural training curricula in Africa, 1982 (E F)
23	Management of group feeding programmes, 1982 (E F P S)
23 Rev. 1	Food and nutrition in the management of group feeding programmes, 1993 (E F S)
24	Evaluation of nutrition interventions, 1982 (E)
25	JECFA specifications for identity and purity of buffering agents, salts; emulsifiers, thickening agents, stabilizers; flavouring agents, food colours, sweetening agents and miscellaneous food additives, 1982 (E F)
26	Food composition tables for the Near East, 1983 (E)
27	Review of food consumption surveys 1981, 1983 (E)
28	JECFA specifications for identity and purity of buffering agents, salts, emulsifiers, stabilizers, thickening agents, extraction solvents, flavouring agents, sweetening agents and miscellaneous food additives, 1983 (E F)
29	Post-harvest losses in quality of food grains, 1983 (E F)
30	FAO/WHO food additives data system, 1984 (E)
30 Rev. 1	FAO/WHO food additives data system, 1985 (E)
31/1	JECFA specifications for identity and purity of food colours, 1984 (E F)
31/2	JECFA specifications for identity and purity of food additives, 1984 (E F)
32	Residues of veterinary drugs in foods, 1985 (E/F/S)
33	Nutritional implications of food aid: an annotated bibliography, 1985 (E)
34	JECFA specifications for identity and purity of certain food additives, 1986 (E F)
35	Review of food consumption surveys 1985, 1986 (E)
36	Guidelines for can manufacturers and food canners, 1986 (E)
37	JECFA specifications for identity and purity of certain food additives, 1986 (E F)
38	JECFA specifications for identity and purity of certain food additives, 1988 (E)
39	Quality control in fruit and vegetable processing, 1988 (E F S)
40	Directory of food and nutrition institutions in the Near East, 1987 (E)
41	Residues of some veterinary drugs in animals and foods, 1988 (E)
41/2	Residues of some veterinary drugs in animals and foods. Thirty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1990 (E)
41/3	Residues of some veterinary drugs in animals and foods. Thirty-sixth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E)
41/4	Residues of some veterinary drugs in animals and foods. Thirty-eighth meeting of the joint FAO/WHO Expert Committee on Food Additives, 1991 (E)

41/5 Residues of some veterinary drugs in animals and foods. Fortieth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1993 (E)
41/6 Residues of some veterinary drugs in animals and foods. Forty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1994 (E)
41/7 Residues of some veterinary drugs in animals and foods. Forty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1994 (E)
41/8 Residues of some veterinary drugs in animals and foods. Forty-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1996 (E)
41/9 Residues of some veterinary drugs in animals and foods. Forty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1997 (E)
41/10 Residues of some veterinary drugs in animals and foods. Forty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1998 (E)
41/11 Residues of some veterinary drugs in animals and foods. Fiftieth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 1999 (E)
41/12 Residues of some veterinary drugs in animals and foods. Fifty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives, 2000 (E)
41/13 Residues of some veterinary drugs in animals and foods. Fifty-forth meeting of the Joint FAO/WHO Expert Committee on Food Additives, 2000 (E)
42 Traditional food plants, 1988 (E)
42/1 Edible plants of Uganda. The value of wild and cultivated plants as food, 1989 (E)
43 Guidelines for agricultural training curricula in Arab countries, 1988 (Ar)
44 Review of food consumption surveys 1988, 1988 (E)
45 Exposure of infants and children to lead, 1989 (E)
46 Street foods, 1990 (E/F/S)
47/1 Utilization of tropical foods: cereals, 1989 (E F S)
47/2 Utilization of tropical foods: roots and tubers, 1989 (E F S)
47/3 Utilization of tropical foods: trees, 1989 (E F S)
47/4 Utilization of tropical foods: tropical beans, 1989 (E F S)
47/5 Utilization of tropical foods: tropical oil seeds, 1989 (E F S)
47/6 Utilization of tropical foods: sugars, spices and stimulants, 1989 (E F S)
47/7 Utilization of tropical foods: fruits and leaves, 1990 (E F S)
47/8 Utilization of tropical foods: animal products, 1990 (E F S)
48 Number not assigned
49 JECFA specifications for identity and purity of certain food additives, 1990 (E)
50 Traditional foods in the Near East, 1991 (E)
51 Protein quality evaluation. Report of the Joint FAO/WHO Expert Consultation, 1991 (E F)
52/1 Compendium of food additive specifications – Vol. 1, 1993 (E)
52/2 Compendium of food additive specifications – Vol. 2, 1993 (E)
52 Add. 1 Compendium of food additive specifications – Addendum 1, 1992 (E)
52 Add. 2 Compendium of food additive specifications – Addendum 2, 1993 (E)
52 Add. 3 Compendium of food additive specifications – Addendum 3, 1995 (E)
52 Add. 4 Compendium of food additive specifications – Addendum 4, 1996 (E)
52 Add. 5 Compendium of food additive specifications – Addendum 5, 1997 (E)
52 Add. 6 Compendium of food additive specifications – Addendum 6, 1998 (E)
52 Add. 7 Compendium of food additive specifications – Addendum 7, 1999 (E)
53 Meat and meat products in human nutrition in developing countries, 1992 (E)
54 Number not assigned
55 Sampling plans for aflatoxin analysis in peanuts and corn, 1993 (E)
56 Body mass index – A measure of chronic energy deficiency in adults, 1994 (E F S)
57 Fats and oils in human nutrition, 1995 (Ar E F S)
58 The use of hazard analysis critical control point (HACCP) principles in food control, 1995 (E F S)
59 Nutrition education for the public, 1995 (E F S)
60 Food fortification: technology and quality control, 1996 (E)
61 Biotechnology and food safety, 1996 (E)
62 Nutrition education for the public – Discussion papers of the FAO Expert Consultation, 1996 (E)
63 Street foods, 1997 (E/F/S)
64 Worldwide regulations for mycotoxins 1995 – A compendium, 1997 (E)
65 Risk management and food safety, 1997 (E)
66 Carbohydrates in human nutrition, 1998 (E S)
67 Les activités nutritionnelles au niveau communautaire – Expériences dans les pays du Sahel, 1998 (F)
68 Validation of analytical methods for food control, 1998 (E)
69 Animal feeding and food safety, 1998 (E)
70 The application of risk communication to food standards and safety matters, 1999 (E)

Availability: September 2000

Ar – Arabic
C – Chinese
E – English
F – French
P – Portuguese
S – Spanish

Multil – Multilingual
* Out of print
** In preparation

The FAO Technical Papers are available through the authorized FAO Sales Agents or directly from Sales and Marketing Group, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy.

This document is one of three publications prepared by the fifty-fourth session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), held in Geneva, in February 2000, and dedicated exclusively to the evaluation of veterinary drug residues in food. The report of the meeting will be published in the WHO Technical Report Series, and the toxicological monographs as No. 45 in the WHO Food Additives Series. Residue monographs in this document provide information on chemical identity, properties, use, pharmacokinetics, metabolism, tissue residues and their depletion, and analytical methods for substances indicated on the cover. This publication is meant for regulatory authorities, veterinary drug researchers and any other concerned persons who wish to gain information on and insights into the assessment of the above-listed information involved in recommending maximum limits for veterinary drug residues in food.

ISBN 92-5-104474-0 ISSN 0254-4725

9 789251 044742

TC/M/X8085E/1/9.00/2000