Residues of some veterinary drugs in animals and foods

FAO
FOOD AND
NUTRITION
PAPER

41/2

Albendazole
Dimetridazole
Diminazene aceturate
Ipronidazole
Isometamidium chloride
Metronidazole
Ronidazole
Sulfadimidine
Sulfathiazole
Trenbolone acetate

FOOD
AND
AGRICULTURE
ORGANIZATION
OF THE
UNITED NATIONS

Residues of some veterinary drugs in animals and foods

Monographs prepared
by the
Thirty-Fourth Meeting of the Joint FAO/WHO
Expert Committee on Food Additives

Geneva, 30 January - 8 February 1989

FAO
FOOD AND
NUTRITION
PAPER

41/2

FOOD
AND
AGRICULTURE
ORGANIZATION
OF THE
UNITED NATIONS
Rome, 1990

The designations employed and the presentation of 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.

M-84
ISBN 92-5-102933-4

TABLE OF CONTENTS

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 30 January - 8 February, 1989

List of Participants

Members invited by FAO

Dr. J. Boisseau, Director, National Laboratory of Veterinary Drugs, La Haute Marche, Javené, Fougères, France

Dr. R. Ellis, Director, Chemistry Division, Food Safety and Inspection Service, United States Department of Agriculture, Washington, D.C. USA (Joint Rapporteur)

Dr. D.P. Kariuki, Director, Veterinary Research Centre, Kenya Agricultural Research Institute, Kikuyu, Kenya (Vice-Chairman)

Dr. R.R. MacKay, Consultant, Hallet Cove, South Australia, Australia

Dr. J.L.R. Martinez, Chief, Toxicology Section, National Centre for Diagnosis and Research in Animal Health, San José, Costa Rica

Dr. T. McEwan, Consulting Chemist, Brisbane, Queensland, Australia

Members invited by WHO

Dr. J. Juskevich, Consultant in Toxicology and Residues Chemistry, Halifax, Nova Scotia, Canada

Dr. B.H. MacGibbon, Consultant in Environmental Health to the Department of Health, London, England (Chairman)

Dr. J.G. McLean, Dean, Faculty of Applied Science, Swinburne Institute of Technology, Hawthorn, Victoria, Australia (Joint Rapporteur)

Professor A. Somogyi, Head, Max von Pettenkofer Institute of the Federal Office of Public Health, Berlin (West)

Dr. D.M. Pugh, Department of Veterinary Surgery and Companion Animal Medicine, Faculty of Veterinary Medicine, University College, Dublin, Ireland

Professor F. Simon, Professor of Pharmacology, Department of Pharmacology and Toxicology, University of Veterinary Sciences, Budapest, Hungary

Secretariat

Dr. R. Benson, US Environmental Protection Agency, Denver, Co, USA (WHO Temporary Adviser)

Dr. S. Brynes, office of New Animal Drug Evaluation, Center for Veterinary Medicine Food and Drug Administration, Rockville, MD, USA (FAO Consultant)

Dr. G. Burin, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (WHO Consultant)

Mr. D. Byron, Joint FAO/WHO Food Standards Programme Group, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, Rome, Italy

Dr. M. Elwell, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA (WHO Temporary Adviser)

Dr. G. Gheorghiev, Joint FAO/WHO Food Standards Programme Group, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, Rome, Italy (Joint Secretary)

Dr. G. Guest, Chairman, Codex Committee on Residues of Veterinary Drugs in Foods, Department of Health and Human Services, Food and Drug Administration, Rockville, MD, USA (WHO Temporary Adviser)

Mr. R.A. Hankin, Administrator, Pharmaceuticals and Veterinary Medicines Division, Commission of the European Communities, Brussels, Belgium (WHO Temporary Adviser)

Dr. Y. Hayashi, Director, Division of Pathology, National Institute of Hygienic Sciences, Tokyo, Japan (WHO Temporary Adviser)

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

Dr. R.J. Heitzman, Science Consultant and Technical Author, Berkshire, England (FAO Consultant)

Dr. F.X.R. van Leeuwen, Laboratory for Toxicology, National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands (WHO Temporary Adviser)

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

Dr. E. Schlede, Max con Pettenkofer Institute of the Federal Office of Public Health, Berlin (West) (WHO Temporary Adviser

Dr. K. Ueda, Director, Department of Veterinary Public Health, Institute of Public Health, Tokyo, Japan (WHO Temporary Adviser)

Dr. J. Weatherwax, Food Quality and Consumer Protection Group, Food Quality and Standards Service, Food Policy and Nutrition Division, FAO, Rome, Italy

Dr. K.N. Woodward, Department of Health and Social Security, London, England (WHO Temporary Adviser)

Dr. M.S. Yong, Human Safety Division, Bureau of Veterinary Drugs, Ottawa, Ontario, Canada (WHO Temporary Adviser)

ABBREVIATIONS USED IN THIS REPORT

ABZ	-	Albendazole
b.w.	-	body weight
c	-	concentration by volume (g/100 ml), (optical rotation only)
D.L.	-	detection limit
EtOAc	-	ethyl acetate
14C	-	radiolabeled carbon - 14
g	-	gram
GC	-	gas chromatography
HPLC	-	High Performance Liquid Chromatography
i.m.	-	intramuscular
i.v.	-	intravenous
kg	-	kilogram
L	-	litre
lbs.	-	pounds
mCi	-	millicuries of radioactivity
max	-	maximum
M	-	molar
mg	-	milligram
ml	-	milliliter
MS	-	mass spectrometry
ND	-	not detected
ng	-	nanogram
nm	-	nanometer
NMR	-	Nuclear magnetic resonance
pg	-	picogram
ppb	-	parts per billion
ppm	-	parts per million
ppt	-	parts per trillion
RIA	-	Radioimmunoassay
s.c.	-	subcutaneous
SD	-	Standard deviation
TBA	-	trenbolone acetate
TBOH	-	trenbolone
TLC	-	thin layer chromatography
^{3}H	-	tritium
UV	-	ultraviolet
WT	-	withdrawal time
w/v	-	weight per unit volume
µg	-	microgram (mcg)
<	-	less than
>	-	greater than

INTRODUCTION

The monographs on the residues of the ten compounds contained in this volume were prepared by the Thirty Fourth Meeting of the Joint FAO/WHO Expert Committee on Food Additives, (JECFA) which was held in Geneva, 30 January-8 February 1989. JECFA has evaluated some veterinary drugs at previous meetings: several antibiotics, including chloramphenicol at the 12th(1) and 32nd(2) meetings and tenbolone acetate and zeranol at the 26th(3), 27th(4) and 32nd(2) meetings. In response to growing concern about mass-medication of food producing animals and its 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(5). Among the main recommendations of this Consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs (CCRVD) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries. At its first session in Washington in November 1986, the newly created CCRVD reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA(6). In response to these recommendations, the Thirty-Second JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods.

The second and third sessions of the CCRVD were held in Washington, D.C. during December 1987 and November 1988 respectively. They revised the priority list of veterinary drugs requiring evaluation. Ten of these drugs were evaluated at the 34th JECFA, which was again devoted only to residues of veterinary drugs in foods.

The present volume contains summary monographs of the residue data on all ten compounds on the agenda. The one anthelmintic was considered in ovine and bovine species including lactating dairy cows. Four 5-nitro-nidazoles were considered. None had been previously evaluated by the Committee. Three of the compounds were considered for turkeys and swine where they are used for treatment and prevention of histomaniasis and dysentery, respectively. The fourth compound, metronidazole, had no data regarding residue depletion studies in food animals and therefore no toxicological evaluation was made. The residue monograph for this substance was based on studies using bacteria, rats and dogs, as well as human data.

Two antimicrobial sulfonamides were considered for swine and sheep with one (sulfadimidine) also considered for milk and eggs produced by treated animals. The growth promoter had been evaluated at three previous meetings of the Committee. The current meeting considered the substance for bovine species and reevaluated the results of three hormonal studies in swine.

The two trypanocides evaluated had not been considered previously by the Committee. Both were considered at the current meeting for ruminants.

The pertinent information in each monograph was discussed and appraised by the whole Committee. The monographs are presented in a uniform format covering chemistry, identity, use, metabolic studies, residue studies and methods of analysis. No attempt has been made at exhaustive literature coverage. More recent publications and documents have been referenced having relevance for the safety assessment of the compounds. In reviewing analytical methods preference has been given to newer, validated procedures with a possible application for control and regulatory purposes. At the end of a monograph an appraisal of the residue data is made in terms of their significance for the safety assessment of the drug.

The assistance of Dr. S. Brynes and Dr. R. Livingston, both of the United States Food and Drug Administration and of Dr. R. Heitzman, a private consultant, in preparing the monographs are 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 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.

3. 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.

4. 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).

5. 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.

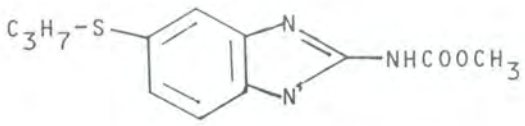
6. Report of the First Session of the Codex Committee on Residues of Veterinary Drugs in Foods. Washington D.C. 27-31 October 1986. ALINORM 87/31.

ALBENDAZOLE

IDENTITY

Chemical name: methyl-5-propylthio-1-h-benzimidazol-2-yl-carbamate

Structural formula:



Molecular formula: $C_{12}H_{15}N_3O_2S$

Molecular weight: 265.3

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Melting Point: 208°-210° C

Solubility: Insoluble in water
Soluble in strong acids and bases
Soluble in dimethyl sulphoxide and acetic acid

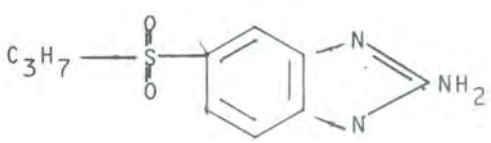
Other properties: White to buff, odourless and stable at room temperature for up to 2 years

MARKER RESIDUE

Chemical name: 5-(propylsulphonyl)-1-H-benzimidazol-2-amine

Other names: Metabolite I, SKB No. 81038

Structural formula:



Molecular formula: $C_{10}H_{13}N_3O_2S$

Molecular weight: 239.3

USE AND DOSE RATES IN FARM ANIMALS AND HUMANS

Farm animals

Albendazole (ABZ) is a parasiticide which is active against the important species of roundworms, tapeworms and flukes in farm animals and humans. The drug is presented in several formulations including drenches, pastes and boluses.

The dosage depends on the target parasite and a withdrawal time is recommended in most situations. The short withdrawal time of 2 days is recommended in Mexico nad the longer time of 30 days is peculiar to Denmark. The drug is not permitted in Italy and the USA.

Animal	Dose (mg per kg)	Withdrawal time (days)	
		Meat	Milk
Cattle	3.8 - 15	2 - 30	1 - 5
Sheep	3.8 - 15	2 - 30	1 - 5

There are several countries which do not allow ABZ for use in lactating or pregnant animals.

Humans

Albendazole is marketed for human medicine in 80 countries. The drug is administered either as tablets or a suspension either as a single dose of 400 mg or 400 mg once daily for 3 days.

Woodward, (1986), states "The limited data available suggest that albendazole has no significant toxic effects at therapeutic doses over a short period of time (up to 5 days)."

PHARMACOKINETICS

Excretion from farm animals

This section covers the clearance rates and routes of excretion of albendazole in target animals. The disposition of residues and metabolites is described in the section on residues.

Absorption of albendazole was moderate in rats and mice with around 20-29% of the dose as albendazole-associated material being found in the urine. Absorption was somewhat higher in cattle and sheep with 54-59% as albendazole/metabolites in the urine (Gyurik et al; 1981).

In a study using 7 calves administered C14-albendazole at 20 mg per kg, 47% of the dose was excreted in the urine by 72 hours. Peak plasma concentrations of radioactivity were observed at 15-24 hours after dosing (SKB Report 24).

In a study using 18 sheep administered C14-albendazole at 16.2 mg per kg, 51% of the dose was recovered in the urine after 120 hours. The peak plasma radioactivity occurred at 15 ours after dosing (SKB Report 23).

In pigs administered C14-albendazole at 16.5 mg per kg, urinary excretion revealed that at least 35% of the dose had been absorbed and 75% of this had been excreted by 48 hours (SKB Report 22).

Discussion

About a half of the dose administered to animals is cleared through the urine in the first 6 days. Thereafter urinary clearance is extremely low. What happens to the other half of

the dose is less clear as no data are available for excretion through the faeces or other possible routes. Even assuming a high residue concentration of 1 ppm in the whole animal at 6 days, this represents only 5-10% original dose, so the missing radioactivity has either been excreted through the faeces and other routes or is still in the stomach/rumen of the animal. This latter idea is not supported by the studies of Marriner & Bogan (1980) and Bogan & Marriner (1983) who showed that albendazole is cleared from the rumen, abomasum and plasma of sheep in about 4 days.

RESIDUES IN ANIMALS AND THEIR EVALUATION

IDENTIFICATION AND QUANTIFICATION OF METABOLITES

Marriner & Bogan (1980) showed that albendazole is not metabolised in the rumen of sheep and suggest that the parent drug is absorbed and then rapidly metabolised by the liver.

Twenty different extraction and purification procedures were used to extract, isolate and identify the metabolites. The samples from animals administered C14-radiolabelled Albendazole were either subjected to enzyme or acid pretreatment and solvent extraction, or, direct solvent extraction. The extracts were assayed by thin layer chromatography using autoradiography or scraping zones and radiocounting. The structures of the metabolites were confirmed using NMR and/or mass spectrometry.

The chemical name, code letter and occurrence in test species is shown in table I.

Parent drug, ABZ, has disappeared from bovine liver by day 6 and was present in day 1 but not day 10 bovine kidneys. No ABZ was found beyond day 2 in ovine liver.

Parent drug, metabolites A and C dominated the profile in bovine liver on day 1 (>75%); I represented <1%. Metabolite A remained fairly constant (about 12%) from day 1 to 12, metabolite C decreased from 38% at day 1 to 14% at day 12. Metabolite I (marker residue) rapidly increased from day 1 and predominated from day 6 through 12. By day 10 only metabolite I was observed in kidney extracts.

The profile in sheep was not dissimilar from that seen in cattle and minor variations are evident in table I.

The metabolic profiles of rats and mice were nearly the same; metabolites C,E,G, and I represented 27, 14, 24 and 15% of the profile in rat urine and 24, 22, 30 and 4% of the profile in mouse urine, respectively.

Metabolic Pathway

The major metabolic pathway in tested species is:

ABZ ——> Sulphoxide (C) ——> Sulphone (A) ——> 2-amino-sulphone (I)

TABLE I. Metabolites of Albendazole

KEY	METABOLITE	TYPE	SPECIES: Cattle L	Cattle K	Cattle U	Sheep L	Sheep U	rat/mouse U	rat/mouse U
A	ABZ SULPHONE	major	+	+	+	+	+	+	+
B	X-HYDROXY ABZ SULPHONE	minor			+		+		
C	ABZ SULPHOXIDE	major	+	+	+	+	+	+	+
D	(unknown)				+		+	+	
E	2-OH-PROPYL-ABZ SULPHONE	minor			+	+	+	+	+
F	CH3-SO-ABZ	minor					+	+	+
G	1-OH-PROPYL-ABZ SULPHONE	minor	+	+		+	+	+	+
H	N-Me-2-AMINO-ABZ SULPHONE	minor			+		+	+	
I	2-AMINO-ABZ SULPHONE	major	+	+	+	+	+	+	+
J	2-AMINO-ABZ SULPHOXIDE	minor	+	+	+	+	+	+	+

(Note: Metabolite I is Marker Residue)

Key:

L is liver, K is kidney, U is urine.
Major is >10% total residue extracted
Minor is <10% total residue extracted
+ is residue detected.

Discussion

The three major metabolites, A, C and I, are a large fraction of the total residues, eg. the Marker Residue (I) is about 20% total residues. Albendazole is a minor component of the residues and cannot be detected within 6 days of withdrawal.

Thus data on the toxicology of the metabolites A, C and I is perhaps of more importance than that of the parent drug. However the major metabolites A, C and I found in the target animals, cattle and sheep, are also present as major metabolites in the laboratory species.

No study of the disposition of metabolites in the pig is available.

EXTRACTION PROCEDURES FOR QUANTIFYING RESIDUES

There are six extraction procedures each giving separate information on the residues. Five of the methods measure C14 activity in residues following the administration of C14-ABZ to animals. The method for measuring the concentration of the marker residue (2-amino-ABZ-sulphone) in tissues from animals treated with non-radioactive ABZ is an HPLC end-point assay after a selective extraction procedure.

Total Residues by C14 Assay

The total radioactive C14 in a sample is measured and converted to ABZ equivalents from the known Specific Activity of the drug.

Total Extractable Residues following Hydrolysis

The homogenised tissue samples are initially hydrolysed with a proteolytic enzyme (Ficin and sometimes Gluculase or Glucarase) followed by heating with strong hydrochloric acid. The mixture is extracted into methanol. The methanol extract is concentrated and extracted into ethyl acetate. The C14 in the extract is measured, (SKB Report 20). Recoveries of C14 spikes are normally >80%. The extracts of liver and kidneys of calves can be separated on TLC for determination of metabolites (see below).

Total Extractable Residues using Ethyl Acetate only

The tissues are homogenised in phosphate buffer (pH 5) and extracted with ethyl acetate. The C14-activity in the extract is measured. Recoveries of C14 spikes are >80%. Extracts of bovine liver and urine can be used for determination of metabolites by TLC (see below).

Metabolite Extraction using TLC

The extracts from above are run on TLC plates. The spots are identified by autoradiography, scraped from the plates and the C14 activity measured in the liver and urine samples. The results for marker residue are shown in table II.

TABLE II. Total Residues, Ethyl Acetate Extractable Residues and Marker Residues in Bovine Liver (ppm)

ANIMAL NUMBER	DOSE (mg/kg)	WT (days)	TOTAL C14 RESIDUES	TOTAL HYDROLYSIS RESIDUES	ETHYL ACETATE EXTRACTABLE	MARKER RESIDUE EtAc-TLC-C14
123	20	1	31.1	27.9	20.4	.192
101	20	4	12.0	2.63	.560	.050
124	20	4	9.83	2.19	.336	.036
105	20	6	7.69	2.38	.374	.100
115	20	6	6.24	1.49	.199	.058
118	20	10	4.13	.786	.153	.056
209	20	10	3.43	.944	.121	.042
90	20	12	4.78	nm	.074	.010
298	20	12	4.91	nm	.068	.015
114	20	20	1.04	.387	nm	nm
148	20	20	1.38	.531	0.64	nm
102	20	30	.481	.108	nm	nm
107	20	30	.401	.078	-	nm

NOTE:

Animal numbers are those referred to in SKB reports. For animals 90 and 298, another report gives total C14 residue values of 4.93 for each. For animals 118 and 209, another report gives total C14 residues of 3.93 and 4.09 respectively.

Animals 90 and 298 occur in R20, T5 and T73 but with slightly different results for Total C14 residues. The values above are from T73; in T5 the values are 4.93 and 4.93.

The values for total residues for animals 118 and 209 are taken from T62 whereas different values of 3.93 and 4.09 are quoted in T73.

nm = not measured
WT = withdrawal time

Marker Residue Determination

The TLC method to measure the C14 Marker Residue is as described previously and the results are given in table II.

An HPLC method is proposed by the sponsor as the regulatory procedure in the USA for measuring concentrations of marker residues in livers of farm animals treated with albendazole in the field.

Liver is homogenised and hydrolysed with 6M HCl at 110^{o}C for an hour. The pH is adjusted to 8 and the hydrolysate extracted with ethyl acetate. The ethyl acetate extract is made acidic (pH 4) and the aqueous phase washed with toluene. The aqueous extract is transferred to a Sepak C-18 cartridge and washed with water and toluene. The marker residue is eluted with ethyl acetate. The eluate is dried and solubilised in methanol for application to HPLC. The peak corresponding to marker residue is detected using a fluorescent detector and quantified against an internal standard.

There is good separation of marker residue peak from matrix background as well as ABZ, other metabolites of ABZ and thiabendazole and it's possible metabolites. Other benzimidazoles and their metabolites were not reported as they are not licensed in the USA. The marker residues are reported in tables IV and V.

Total residues

The total residues in edible tissues of cattle were determined using C-14-albendazole. The results when doses of 10,15 or 20 mg per kg body weight were administered to cattle are shown in table III.

TABLE III. Total Residues in Tissues and Plasma following Sigle Dose of C14-Albendazole as an Oral Capsule to Cattle (ppm)

DOSE: 20 mg per kg to CALVES (Data taken from SKB Report 24)

WT DAYS	MUSCLE	LIVER	KIDNEY	FAT	PLASMA
1	7.90	29.0	21.7	.40	5.49
4	.07	8.20	4.40	.04	.96
6	.06	6.76	3.19	.02	.69
10	.05	3.57	1.93	.01	nm
20	.03	1.15	.63	<.01	nm
30	.02	.42	.25	<.01	nm

DOSE: 15 mg per kg to CALVES (Data from SKB Report 61)

WT DAYS	MUSCLE	LIVER	KIDNEY	FAT
1	4.83	22.5	15.6	1.76
4	.06	5.98	2.15	.21
6	.04	4.33	1.6	.08
12	nd	2.47	.85	.07
14	.03	1.84	.98	.03
20	.02	1.21	.41	.04

DOSE: 10 mg per kg to COWS (Data from SKB Report 62)

WT DAYS	MUSCLE	LIVER	KIDNEY	FAT
60	.010	.279	.062	.005
90	.011	.106	.029	.004
120	.008	.090	.028	.002
150	.006	.045	.020	.002
180	.006	.026	.019	.002

Note: WT = withdrawal time
nm = not measured
nd = not detected

MARKER TISSUE AND MARKER RESIDUE

The United States Food and Drug Administration has developed the use of a "marker tissue" and a "marker residue" to provide information on residues in target species. The "marker tissue" is the last tissue to deplete to its permitted concentration. The "marker residue" is the residue which depletes in a known relationship to the total residues in the marker tissue.

The 2-amino-ABZ-sulphone (Metabolite I) is selected as the "marker residue" because after day 4 of withdrawal of drug the concentration of I in bovine liver as a percentage of the total concentration of residues is fairly constant at about 20% (see table IV).

TABLE IV. Marker residue in Cattle Liver (ppm)

DOSE: 15 mg per kg

WT DAYS	TOTAL RESIDUES	MARKER RESIDUE	% TOTAL AS MARKER RESIDUE
4	6.41	1.01	17.0
6	4.71	.87	18.5
6	3.95	.64	16.1
12	2.55	.47	18.4
14	1.69	.30	17.5
40	0.34	.07	20.3

The data was taken from a table in SKB Report 1. In another table of the same report, evidence is presented showing that in 16 cattle given 10 mg per kg albendazole and sampled from 20 to 180 days after administration the mean value and standard deviation for the marker residue as a percentage of total residues was 19.3 $\pm$ 3.1%.

There is an abundance of information (see table V) in the SKB reports which allow a straight-forward choice of liver as the marker tissue.

The concentrations of marker residue in bovine liver were determined in cattle administered albendazole in four different formulations. The results in Table V show that the residues in liver were not very different between the type of formulation used.

TABLE V. Concentrations of Marker Residue in Liver of Cattle Treated with Different Formulations of Albendazole at Single Dose of 10 mg per kg b.w (ppm)

WT DAYS	BOLUS	SUSPENSION	PREMIX	PASTE
12	.364	nm	.307	nm
16	.227	nm	.273	nm
20	.146	.131	.201	.148
24	.146	.113	.137	.100
28	.115	.078	.101	.081
32	.079	.052	.086	.064

Data sources were SKB Reports 64, 65, 66 and 67 respectively. SKB Report 10 indicates about 0.90 ppm total 14C residues.

nm = not measured
WT = withdrawal time

There are insufficient data to make comparisons with the concentration to the marker residue and the total residues at this dose and these WTs. This is because the cattle are dosed with non-radioactive ABZ.

Interestingly, the parent drug is found as a residue during the first few days after withdrawal of the drug but then is not detectable. Detection of both marker residue and parent drug may be a regulatory tool for monitoring early stages in the withdrawal period. Under most allowed uses parent drug should never be detected as a residue at the recommended withdrawal times.

REFERENCE METHODS FOR RESIDUES

METHOD FOR MARKER RESIDUE

The HPLC method described previously is specific for measuring Marker Residue in liver tissue of cattle dosed with albendazole. Thiabendazole and its metabolites do not interfere in the assay. Thiabendazole was chosen because it was the only benzimidazole licensed in the USA at that time, however no evidence is presented as to whether other benzimidazole drugs (e.g. Fenbendazole, oxfendazole) which are widely used outside the USA, interfere with the HPLC assay. It is also not clear whether the method can be used with tissues other than liver.

Extraction Procedure

The HPLC procedure for marker residue is different from those used to determine the extractable residues in that the Marker Residue is almost exclusively extracted. The difference is due to the use of more selective partitioning at a strict pH and the use of a Sepak C18 cartridge for the final purification step. The eluate from the Sepak column containing the Marker Residue can be split into two aliquots, one aliquot is used for HPLC determination and the other aliquot can be used for mass spectrometry in the confirmatory method (see below).

Table VI shows the typical distribution of residues in a bovine liver which might be found at withdrawal intervals beyond the first week after treatment.

TABLE VI. Cow dosed 20 mg per kg b.w.; liver collected after 20 days withdrawal time

	ppm	% total
Total C14 residues in unextracted tissue	0.900	100
C14 residues in liver extract prior to HPLC	0.199	22
Marker Residue in extract by HPLC	0.178	19.8

Confirmatory Method for Marker Residue

Marker Residue, Metabolite I, is extracted as described for the HPLC method and its presence confirmed by gas chromatography/mass spectrometry using multiple ion detection of the t-butyldimethylsilyl (t-BDMS) derivatives (SKB Reports 11 and 12).

The GC/MS confirmation of Marker Residue in cattle liver consists of:

- identifying a response at the retention time on the GC corresponding to the Residue-BDMS derivative.
- this response contains the four characteristic mass ions (m/e = 189, 354, 410 and 467)
- the relative ion intensity for each ion is reproducible within 10% of that obtained for derivatised control cattle liver extracts fortified with marker residue chemical.

BIOAVAILABLE RESIDUES

Introduction

There are significant amounts of residues in edible tissues of meat animals administered albendazole. The total residues in liver are not only the highest after 1 and 2 days withdrawal time but are also very different from those measured from 4 or 6 days onwards after withdrawal of the drug. There are also considerable differences between the nature of the residues in the livers of cattle and sheep.

The residues in sheep liver at 1 day are almost wholly extractable with ethyl acetate and at 2 days 53% of the residues are ethyl acetate extractable. There is a steady fall in the percentage of residues which are "extractable" to a plateau of about 13% 8 days after withdrawal.

52% of the total residues in cattle liver sampled at 1 day withdrawal time are extractable with ethyl acetate. However the "extractable" residues measured in samples taken 4-20 days after withdrawal of drug are 1.1 - 3.2% of the total residues. Also at the 1 day withdrawal time the parent drug albendazole is found as a major (27% total residues) residue but is not found at the later withdrawal times.

In measuring the bioavailability of the residues, the tissue was chosen where the percentage "extractable" residues was lowest, i.e. 1.1% in liver from two calves sampled 12 days after administering 20 mg C14-albendazole per kg body weight. The bioavailability of residues in the kidneys of these treated animals and two other calves was also measured.

Bioavailability Studies

Three studies were carried out (SKB Reports 68 and 69) in which powdered liver or kidney containing C14-residues of albendazole were fed for 24 hours to rats. The excretion of radioactivity into bile, urine and residual radioactivity in liver measured.

The studies are satisfactory for liver tissue but there was a wide variation in the results for the kidney studies. The liver powders are derived from two calves (90,298) and the kidney powders from four calves (90, 118, 209, 298). The overall means suggest that the results for both tissues are similar. They are summarised in table VII.

TABLE VII. Bioavailability studies using powdered calves tissue

Powdered Tissue	Average percentage of initial dose in Urine	Bile	Liver	G.I. Tract	Faeces	Recovery total
Liver	2.6	nm	.05	.06	90.4	93.1
Liver*	3.0	1.2	0.0	1.0	97.4	102.6
Liver	3.5	1.8	.03	2.8	83.5	91.6
Kidney	2.8	nm	.10	nm	89.9	92.8

n = 13 SD = 0.7

* One value excluded because of loss of part of sample in transit.

nm = not measured

Comment

The bioavailability of residues in liver of calves at 12 days after the administration of 20 mg albendazole per kg b.w. could be considered as <10% and probably <5%.

The bioavailability of residues in kidneys of calves may be similar to those in liver tissue but the results are more variable.

No data is presented for the bioavailability of residues in sheep tissues, which contain a higher percentage of ethyl acetate extractable residues than calf liver.

Although it is a reasonable supposition there is no confirmation of the theory that bioavailable residues are the same as ethyl acetate extractable residues.

RESIDUES AND TOLERANCES

Introduction

Whereas some countries (e.g. USA & Italy) have not approved the use of ABZ others have recommended tolerances of between 0.5 mg per kg (e.g. Germany) and 5 mg per kg (e.g. UK).

The residue data shown in table III clearly indicate that high (up to 30 mg per kg) concentrations of total residues are present during the first few days after administration of drug. These residues are readily extractable and could be considered bioavailable.

The total residues are highest in the liver and kidney and fall gradually in cattle to about 1 mg per kg after 20 days withdrawal time. However by about day 10 most of the residues are not extractable with ethyl acetate suggesting a large percentage of bound residues which are not bioavailable. The total residues in the main edible tissues, muscle and fat are high on day 1 but fall very rapidly to levels <0.1 mg per kg by day 4.

A difficulty with the data is the variety of extraction procedures used to obtain the residue data. Whereas it is obvious when total residues were measured by the use of C14-ABZ, the further fractionation of the residues needed careful examination. The use of hydrolytic enzymes and/or acid hydrolisis liberate substantial amounts (20% - 90%) of ethyl acetate extractable material.

On the other hand a smaller fraction of C14-residues are extracted by using ethyl acetate alone. Of this extractable fraction <1% is marker residue on day 1 and 9 to 36% is marker residue between days 4-12.

Liver is chosen as the marker tissue for USA purposes and the main metabolite, 2-amino-ABZ-sulphone, is selected as the marker residue in liver. This is because the liver has both the highest and most persistent residues and the amino-sulphone is a reasonably constant fraction (about 20%) of the residues. However the residues are determined by different extraction procedures from the radiometric studies.

The procedure for measuring the marker residue is carried out with acid hydrolysis, ethyl acetate extraction, Sepak column chromatography and HPLC end-point determination. Thus the measurement of marker residue is an indicator of total residues and not the readily extractable residues as discussed above. Unfortunately no studies were reported for determining the best marker residue for the other edible tissues (muscle, kidney and fat). Thus there is no method for estimating total residues in these other tissues.

CAPTEC - SLOW RELEASE CAPSULES

Introduction

SKB Report 70 describes the level of ethyl acetate extracted residues in sheep administered one or two intraruminal ABZ capsules (CAPTEC). Each capsule releases about 17.5 mg ABZ per day which in an adult sheep is a rate of about 0.5 mg ABZ per kg sheep per day.

In another study C14-ABZ was infused into sheep at a rate of 0.5 mg per kg per day for either 7 or 14 days. This experiment was designed to simulate the release of ABZ from the Captec capsules and provided information on total and specific residues.

Experimental

Infusion with C14-ABZ

Four sheep were administered through an intraruminal catheter 0.5 mg C14-ABZ per kg body weight for 7 days. Two of the sheep were slaughtered and the infusion was continued in the remaining two sheep for a further 7 days and then slaughtered. During the infusion periods samples of plasma, urine and faeces were collected at regular intervals. At slaughter tissues were collected and deep frozen to await analysis.

The samples were analysed for total C14 residues and for the concentrations of the 3 main metabolites, the ABZ-sulphoxide (C), ABZ-sulphone (A) and the 2-amino-ABZ-sulphone (I).

Dosing with CAPTEC ABZ capsules

Sixty four sheep were divided into two groups. One group received one CAPTEC capsule and the other group were dosed with two CAPTEC capsules. Four sheep from each group were slaughtered at 5, 10, 25, 54, 74, 90, 96 and 98 days after dosing. Samples of muscle, liver, kidney and fat were collected and stored deep frozen until analysis.

The concentration of two metabolites, ABZ-sulphoxide, ABZ-sulphone were measured by extraction with ethyl acetate and HPLC. The concentration of 2-amino-ABZ-sulphone was measured by the method for measuring the concentration of Marker Residue (see section 6).

Results

C14 Infusion

There was no significant difference between the total residues of C14-ABZ in the edible tissues sampled at the end of either 7 or 14 days continuous infusion. This would indicate that equilibrium was reached after 7 days. The totals are shown in table VIII.

TABLE VIII. Total C14-ABZ residues (ppm) following infusion

Sheep number	Days Infusion	Tissue C14 concentration (PPM) Muscle	Liver	Kidney	Fat
391	7	0.16	2.34	0.64	0.05
472	7	0.12	1.84	0.63	0.04
383	14	0.20	2.18	0.92	0.07
457	14	0.12	2.33	0.49	0.03

The concentrations (ppm) of the residues of the C14-metabolites in muscle, liver and kidney are given in table IX. The three metabolites have some similarities to the pattern seen in animals treated orally with ABZ and measured on the first day after dosing, i.e. the sulphone and sulphoxide are the major metabolites whereas the 2-amino-sulphone is a minor metabolite in samples taken near to time of dosing. Dosing in this case is of course continuous. It is surprising that the residue of the parent drug was not followed as this could be expected to be an important residue while the drug is being absorbed.

TABLE IX. Metabolite residues (ppm) following infusion

Metabolite	Muscle 7days	Muscle 14days	Liver 7days	Liver 14days	Kidney 7days	Kidney 14days
ABZ-sulphone	0.06	0.07	0.49	0.50	0.12	0.18
ABZ-sulphoxide	0.11	0.06	0.54	0.70	0.13	0.28
2-Amino-ABZ-sulphone	<LD	<LD	0.06	0.10	0.05	0.06
sum of metabolites as % Total C14 residues	121	81	52	58	47	74

On the last day of infusion the % dose of C14 excreted into the urine was 62.1% (7day infusion) and 61.7% (14day infusion) and into the faeces was 25.2% (7day infusion) and 20.2% (14day infusion). <LD = lower limit of detection.

CAPTEC dosing

28 of 32 sheep treated with 1 Captec and 24 of 32 sheep receiving 2 Captecs were analysed for residues. No record of the missing sheep is given in the report.

The measurement of metabolite I (Marker Residue) by the HPLC method is different from that used for the other two metabolites and the extraction procedure for the marker residue liberates more extractable residue. Thus the relationship between the concentrations of the three metabolites is unsound, it is possible that the metabolites A and C would give much higher concentrations if they were liberated by hyrolysis. On the other hand the concentration of I would be lower if it had been measured in a mild ethyl acetate extract similar to that used for the other two metabolites.

The concentration of the three metabolites of ABZ are shown in table X. The sulphoxide is the most abundant metabolite in liver whereas the 2-amino-sulphone emerges as a main metabolite in liver and muscle as the time after dosing extends beyond 2 months.

Each capsule is designed to release 17.5 mg ABZ per day and this zero order release rate should result in a constant level of residues. This is clearly not the case because the concentration of the metabolites decreases with time. Either the metabolism of the sheep has changed giving rise to different unidentified residues or the capsule does not release ABZ at a constant rate. Further information from SKB providing evidence from another experiment showed that the release of drug from the capsule is linear over a 93 day period. Thus the metabolism of the drug must be altered during this period.

TABLE X. Residues (ppm) of metabolites after dosing sheep with CAPTEC

1 CAPTEC per sheep

Days after dosing	Muscle C	Muscle A	Muscle I	Liver C	Liver A	Liver I
5	ND	0.09	0.02	0.85	0.68	0.30
10	ND	0.06	0.02	0.88	0.96	0.21
25	ND	0.08	0.01	0.88	0.54	0.15
54	ND	ND	0.01	0.47	0.35	0.25
74	ND	ND	<0.01	0.31	0.22	0.22
90	ND	ND	0.01	0.05	0.03	0.03
96	ND	<0.06	0.01	<0.33	<0.36	0.08
98	ND	ND	ND	ND	ND	0.04
2 CAPTECS per sheep						
5	0.09	0.22	0.03	1.43	1.12	0.67
25	0.07	0.14	0.02	1.14	0.90	0.11
54	ND	<0.04	<0.02	0.84	0.63	0.36
98	ND	ND	ND	ND	<0.02	0.05

ND = not detected

Comment

There is insufficient information to properly appraise the residue patterns and concentrations following the use of the CAPTEC capsules.

The constant release of drug from the capsules over a 100 day period was not presented in report 70 but new information suggests that the drug is released at a near constant steady rate for at least 93 days.

The results using C14-ABZ infusion do not necessarily simulate the release of ABZ from the CAPTEC devices.

The residues of the metabolites A and C were measured in ethyl acetate extracted material and the concentration of I was measured by a more exhaustive extraction procedure. The concentrations represent an unknown proportion of the total residues.

FINAL COMMENTS

A large amount of information is available for the nature and disposition of residues of albendazole in cattle. There is less information for residues in sheep and very little information for other species.

The metabolites of albendazole found in cattle and sheep are also found to a greater or lesser degree in rodents. There are 3 or 4 key metabolites and metabolite I, 2-amino-ABZ-sulphone, is the most important because it can be declared a "Marker Residue" using FDA, USA guidelines.

The parent drug, albendazole, is only found as a residue in edible tissues within a few days of administration and should not be detected in farm animals treated according to the recommended withdrawal periods observed for albendazole by most countries.

The total residues in edible tissues of cattle are highest at 1 day withdrawal time (WT) with the highest concentrations in the metabolising organs, the liver and kidney. The concentrations decline to <0.1 mg per kg in muscle and fat and <5 mg per kg by 10 days withdrawal time and to <1 mg per kg levels within 30 days withdrawal time. The residues remain at detectable levels for at least 6 months in some tissues.

The SKB reports describe a large number of methods for measuring and identifying the residues. Only two methods give a reliable measurement of the total residues. One method is a radiometric method using C14-albendazole and can only provide information for animals dosed with radioactive albendazole. The other is the method for measuring the marker residue where it has been shown that the marker residue in cattle at or beyond 4 days WT, is a constant fraction, about 20%, of the total residues measured by the radiometric method . Thus an estimate of the total residues in liver (the "Marker Organ") is possible. It is interesting that the marker residue is only about 1% total residues on 1 day WT in cattle and many cattle sampled at this time would give a false and low estimate of total residues in liver, probably indicating 1-2ppm instead of the true 30ppm. No marker residue was examined for estimating total residues in muscle, kidney or fat.

Other methods for measuring residues are used to define the nature of the residues. Strong hydrolysis of the tissues combined with organic solvent extraction can liberate the majority of the residues. However if only ethyl acetate is used to extract the tissues very little (<5%) of the residues are extracted in tissues sampled from 4 days WT onwards in cattle. About one half of the residues are extracted by ethyl acetate at 1 day WT. The residues extracted with ethyl acetate are referred to as "free" residues by SKB.

The pattern of free to bound residues in sheep is different compared with cattle. In sheep tissues a much higher proportion of the residues are extractable with ethyl acetate.

It may be wise not to think that the ethyl acetate extractable residues are either the free fraction or that they can be equated with the bioavailable fraction. However the bioavailability of the residues in bovine liver and kidney has been measured. The experiments were carried out by feeding rats with tissue samples with the lowest fraction of ethyl acetate extractable material. The bioavailability of the residues was definitely <10% and probably <5%. The results for liver showed much less variation than the results for kidneys. No bioavailability studies were carried out on sheep tissues even though they have a higher free to bound ratio of residues than cattle.

REFERENCES

SKB Reports 1 - 70. Smith, Kline & Beckman reports on Albendazole. These are reports submitted to regulatory authorities and are confidential.

Woodward K.N. (1986). Toxicity review of some benzimidazoles anthelmintics. DHSS, UK.

Gyurik, R.J., Chow, A.W., Zaber, B., Brunner, E.L., Miller, J.A., Villani, A.J., Petka, L.A. and Parish, R.C. (1981), Metabolism of albendazole in cattle, sheep, rats and mice. Drug metab. and Disposition, 9, 503-508.

Bogan J.A. and Marriner, S.E. (1983) Pharmacokinetics of Fenbendazole, Albendazole and Oxfendazole in sheep. Vet. Res. Communications. 7, 271.

Marriner, S.E. and **Bogan, JA** (1980), Pharmacokinetics of Albendazole in Sheep. A.J. Vet. Res 41, 1126-1129.

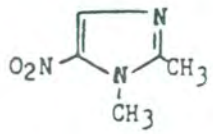
DIMETRIDAZOLE

IDENTITY

Chemical name: 1,2-dimethyl-5-nitroimidazole

Synonyms: Emtryl
Emtrymix
Emtrylvet
Unizole

Structural formula:



Molecular formula: $C_5H_7N_3O_2$

Molecular weight: 141.13

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: needles from water

Melting point: 138-139°C

(Windholz, 1983)

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Dimetridazole is used in turkeys (1) for the prevention and treatment and as an aid in the control of blackhead (histomoniasis, infectious enterohepatitis), (2) for growth promotion, and (3) for improved feed efficiency. The drug is also effective in the prevention and treatment of swine dysentery.

Dosages

Dimetridazole is administered to poultry and swine primarily through feed or water in concentrations normally ranging from 0.01% to 0.06%. Dimetridazole, as the methane sulfonate, may also be administered to swine via injection in a dose that is similar to that delivered through feed or water.

RADIOLABELED RESIDUE DEPLETION STUDIES

Turkeys

Two turkeys weighing approximately 3 kg were administered a single dose of 32 mg/kg (equivalent to a daily dose of 0.05% in drinking water) dimetridazole labeled with 14C in

the 2-position of the ring and the 2-methyl group. The birds were sacrificed 72 hours after treatment and the residue in tissues was determined by both a radiochemical and polarographic method. For the samples analyzed, residue concentrations were below the limit of detection of the assays (polarographic method, <0.05 for muscle, liver and skin, <0.1 for kidney; radiochemical method, <0.03 for liver and <0.05 for kidney). It should be noted that the radiochemical determination for tissues was made on the benzene extract of tissue, not on the whole tissue. (Law, et al., 1962; Law, et. al., 1963)

Swine

Dimetridazole, labeled with 14C in the N-methyl group, was administered to two pigs in a single dose of 29.8 mg/kg or 16.6 mg/kg. The animals were sacrificed 6 or 17 hours after dosing, respectively. Concentrations of total residue in muscle (foreleg), liver, kidney and fat are given in Table I. (Mulcock and Unsworth, 1973a)

Table I. Total Residue in Tissues of Swine Treated With 14C-Labeled Dimetridazole (ppm)

Withdrawal Time	Muscle	Liver	Kidney	Fat
6 hours	8.59	15.40	36.05	3.60
17 hours	0.42	3.00	1.48	*

* not measured

Four pigs weighing 12 to 22 kg were treated with a single dose of approximately 25 mg/kg (actual range 19 to 37 mg/kg) of dimetridazole labeled with 14C in the N-methyl group. Muscle biopsies were taken from three pigs at 24 and 48 hours after treatment and from one pig at 72 hours after treatment. Animals were sacrificed 7 days after administration of labeled dimetridazole and samples of muscle, liver, kidney and fat were analyzed. The mean concentrations of total residue of dimetridazole measured in the biopsies and in the edible tissues at slaughter are given in Table II. (Unsworth, 1972)

Table II. Total Residue in Tissues of Swine Treated With 14C-Labeled Dimetridazole (ppm)

Withdrawal Time (hours)	Biopsy Muscle	Muscle	Liver	Kidney	Fat
24	0.67	—	—	—	—
48	0.27	—	—	—	—
72	0.40	—	—	—	—
168	—	0.32	0.91	0.81	0.37

RESIDUE DEPLETION STUDIES

Chickens

The concentrations of dimetridazole were determined in the edible tissues of chickens dosed at 0.025% or 0.05% through the feed or drinking water. Analyses were conducted with a polarographic method with a limit of detection of 0.1 ppm. Mean residue levels of dimetridazole in tissues at various withdrawal times are given in Table III. (Muggleton, 1963)

Table III. Mean Concentrations of Dimetridazole in Chicken Tissues (ppm)

Tissue	Withdrawal Time (days) 0			1			2		
	A	B	C	A	B	C	A	B	C
muscle	2.9	<0.1	0.4	<0.1	<0.1	*	<0.1	<0.1	*
liver	1.7	<0.1	0.5	<0.1	<0.1	*	<0.1	<0.1	*
kidney	0.5	<0.1	0.1	<0.1	<0.1	*	<0.1	<0.1	*
skin	1.8	<0.1	0.1	<0.1	<0.1	*	<0.1	<0.1	*

A = dosed for 6 days in drinking water at 0.05%
B = dosed for 14 days in the feed at 0.025%
C = dosed for 14 days in the feed at 0.05%

* no samples

Turkeys

Turkeys were treated with 0.025%, 0.05%, 0.1% or 0.2% dimetridazole in the feed until they were 24 weeks of age. For the 0.025% and the 0.05% groups, birds were sacrificed at 0, 3, 6, 12, 24 and 48 hours after withdrawal of medicated feed. For the 0.1% and 0.2% groups, the birds were sacrificed at 0, 3, 6, 12, 24, 48, 72 and 96 hours. Tissue samples of breast muscle, liver, kidney, fat and thigh skin were taken at sacrifice. The concentration of dimetridazole in tissues was determined using a polarographic method with a claimed limit of detection of 0.05 ppm. The results are given in Tables IV and V. (Condren, et al., 1963)

Table IV. Concentration of Dimetridazole in Turkeys (ppm)

Tissue	Drug Level(%)	Withdrawal Time (hours) 0	3	6	12	24	48
muscle	0.05	3.44	1.98	0.71	0.92	ND	<.05
	0.025	0.10	0.09	0.08	ND	<.05	ND
liver	0.05	6.67	1.17	2.34	0.10	<.05	ND
	0.025	0.12	<.05	0.12	<.05	<.05	ND
kidney	0.05	0.64	0.11	0.06	0.08	<.05	ND
	0.025	0.15	0.05	<.05	<.05	<.05	ND
fat	0.05	2.27	1.31	0.75	0.89	0.08	<.05
	0.025	0.12	<.05	0.05	<.05	0.05	<.05
skin	0.05	3.28	1.29	1.10	0.78	0.06	ND
	0.025	0.06	0.08	0.12	<.05	<.05	ND

ND = No detectable residue

Table V. Concentration of Dimetridazole in Turkeys (ppm)

Tissue	Drug Level (%)	Withdrawal time (hours) 0	3	6	12	24	48	72	96
muscle	0.1	11.56	5.75	5.24	3.31	0.22	0.08	0.05	0.06
	0.2	12.72	12.40	8.66	0.29	1.04	0.23	*	0.06
liver	0.1	15.20	6.52	6.96	4.75	0.48	<.05	<.05	0.06
	0.2	14.88	14.68	8.50	0.21	1.07	0.24	*	0.16
kidney	0.1	6.80	0.88	1.65	1.42	0.10	<.05	<.05	<.05
	0.2	17.76	12.44	6.75	0.90	0.14	0.14	*	0.08
fat	0.1	7.40	3.41	2.99	1.81	0.10	<.05	0.08	<.05
	0.2	*	0.03	2.69	*	0.69	0.29	*	0.11
skin	0.1	7.40	3.90	3.84	3.12	0.26	0.10	0.07	0.06
	0.2	15.68	6.60	6.67	0.27	0.75	0.22	*	0.10

* Insufficient sample or birds not available at this withdrawal time

Turkeys were given free access to medicated water (0.05%) for 6 days. Samples of tissues were assayed with a polarographic method with a level of sensitivity of 0.1 ppm. The results are given in Table VI. (Law, et al., 1962)

Table VI. Concentration of Dimetridazole (ppm) in Tissues of Turkeys Treated for 6 Days with 0.05% Dimetridazole in Drinking Water

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin
0	0.92	0.68	<0.1	0.38
1	0.04	0.22	<0.1	<0.1
2	<0.1	<0.1	<0.1	<0.1

A group of 20-week old turkeys was treated with 0.08% dimetridazole in the feed (therapeutic dose) for 7 days. Another group of 10-week old turkeys was treated with 0.02% dimetridazole in the feed (prophylactic dose) for 10 weeks. At the end of the dosing period for each group, six birds were sacrificed at each of the following withdrawal times: 0, 1, 2, 3, 5, 7, 10, and 14 days. Muscle, liver, kidney and skin were collected from each bird at the time of sacrifice. Tissue samples were assayed for dimetridazole using a gas chromatographic method with a limit of detection of 2 ppb. The results are presented in Tables VII and VIII. (DHHS, 1986)

Table VII. Dimetridazole in Tissues of Turkeys Treated With 0.08% Dimetridazole in the Feed (ppb)

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin
0	168	9.2	<2	170
1	<2	<2	<2	4.3
3	<2	<2	<2	<2
5	<2	<2	<2	<2

Table VIII. Dimetridazole in Tissues of Turkeys Treated With 0.02% Dimetridazole in the Feed (ppb)

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin
0	125	<2	<2	145
1	<2	<2	<2	2.5*
2	<2	<2	<2	3.7*
3	<2	<2	<2	3.0*
5	<2	<2	<2	2.5*
7	NS	NS	NS	2.6*

NS = not sampled
* samples thought to be contaminated

Swine

Numerous residue depletion studies have been conducted in swine. Many of these, however, report residue levels for parent dimetridazole determined with a dc polarographic assay with a limit of detection of 0.1 ppm. As discussed under the **Metabolism** section that follows, the metabolite 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI) was found to be the major identifiable component of the residue in muscle and kidney of pigs treated with dimetridazole. For this reason, analytical methods capable of monitoring for HMMNI as well as parent dimetridazole in the very low ppb range were developed. Therefore, only residue depletion studies relying on the improved analytical methodologies are noted.

Pigs approaching market weight were medicated with dimetridazole in the drinking water at 0.02% for 5 days. Groups of three pigs were sacrificed at various withdrawal times and samples of tissues were analyzed with a differential pulse polarographic assay with a limit of detection of 2 ppb. The results of this study are presented in Table IX. (Craine and Anderson, 1973a; Craine, et al., 1974)

Table IX. Residues in Tissues of Pigs Medicated With 0.02% Dimetridazole in Drinking Water (ppb)

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin	Fat
0	301	<2	235	123	25
3	<2	*	<2	*	*
5	<2	*	<2	*	*
6	<2	<2	<2	<2	<2
7	<2	*	<2	*	*

* not analyzed

Pigs were medicated with dimetridazole in the feed at 0.24% (approximately 20 times the proposed dosing level) for 14 days. Groups of three pigs were sacrificed at various withdrawal times and samples of tissues were analyzed with the differential pulse polarographic method noted above. The results are summarized in Table X. (Craine and Anderson, 1973b)

Table X. Residues in Tissues of Pigs Medicated With Dimetridazole at a Level of 0.24% in the Feed (ppb)

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin	Fat
0	4119	4	3137	2373	754
1	4	<2	4	12	<2
2	<2	<2	<2	4	<2
3	<2	*	<2	<2	*
4	<2	*	<2	<2	*

* not analyzed

Pigs were medicated with dimetridazole in the feed at 0.0125% for at least 30 days. Groups of three pigs were sacrificed at various withdrawal times and samples of tissues were assayed with the differential pulse polarographic method having a detection limit of 2 ppb. The results of this study are given in Table XI. (Craine and Anderson, 1973c)

Table XI. Residues in Tissues of Pigs Medicated With Dimetridazole at a Level of 0.0125% in Feed (ppb)

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin	Fat
0	261	<2	168	147	53
1	<2	<2	<2	<2	<2
2	<2	<2	<2	<2	<2
3	<2	*	<2	*	*
4	<2	*	<2	*	*
5	<2	*	<2	*	*

* not analyzed

Five female piglets, 2-3 months of age, were medicated with dimetridazole at a level of 0.031% in the feed for 14 days. At 2, 6, 12, 25 and 49 hours post dosing one animal was sacrificed and samples of muscle, liver, kidney, fat and skin were collected. Tissues were analyzed for HMMNI and dimetridazole with an HPLC procedure with electrochemical detection. The method has a claimed limit of detection of 0.5 ppb. The results of this study are summarized in Table XII. (Sved and Carignan, 1987)

Table XII. Residues in Tissues of Piglets Medicated With Dimetridazole at a Level of 0.031% in the Feed (ppb) (ND = not detected)

Withdrawal	Muscle		Liver		Kidney	
Time (hrs)	HMMNI	DMZ	HMMNI	DMZ	HMMNI	DMZ
2	500	20	0.9	ND	92	1.7
6	100	1.3	0.2	0.2	6.7	ND
12	3.2	ND	ND	ND	0.7	ND
25	ND	ND	ND	ND	ND	ND
49	ND	ND	ND	ND	ND	ND

METABOLISM STUDIES

Turkeys

Three days after a single oral dose of labeled dimetridazole containing 14C in the 2-methyl group and the 2-position of the ring to turkeys at 32 mg/kg, an average of 79.4% of the radioactivity was recovered in the urine, 8% in the feces, and 1.2% in the expired air. Approximately 90% of the administered drug, therefore, can be accounted for in the three-day period after dosing. (Law, et al., 1962)

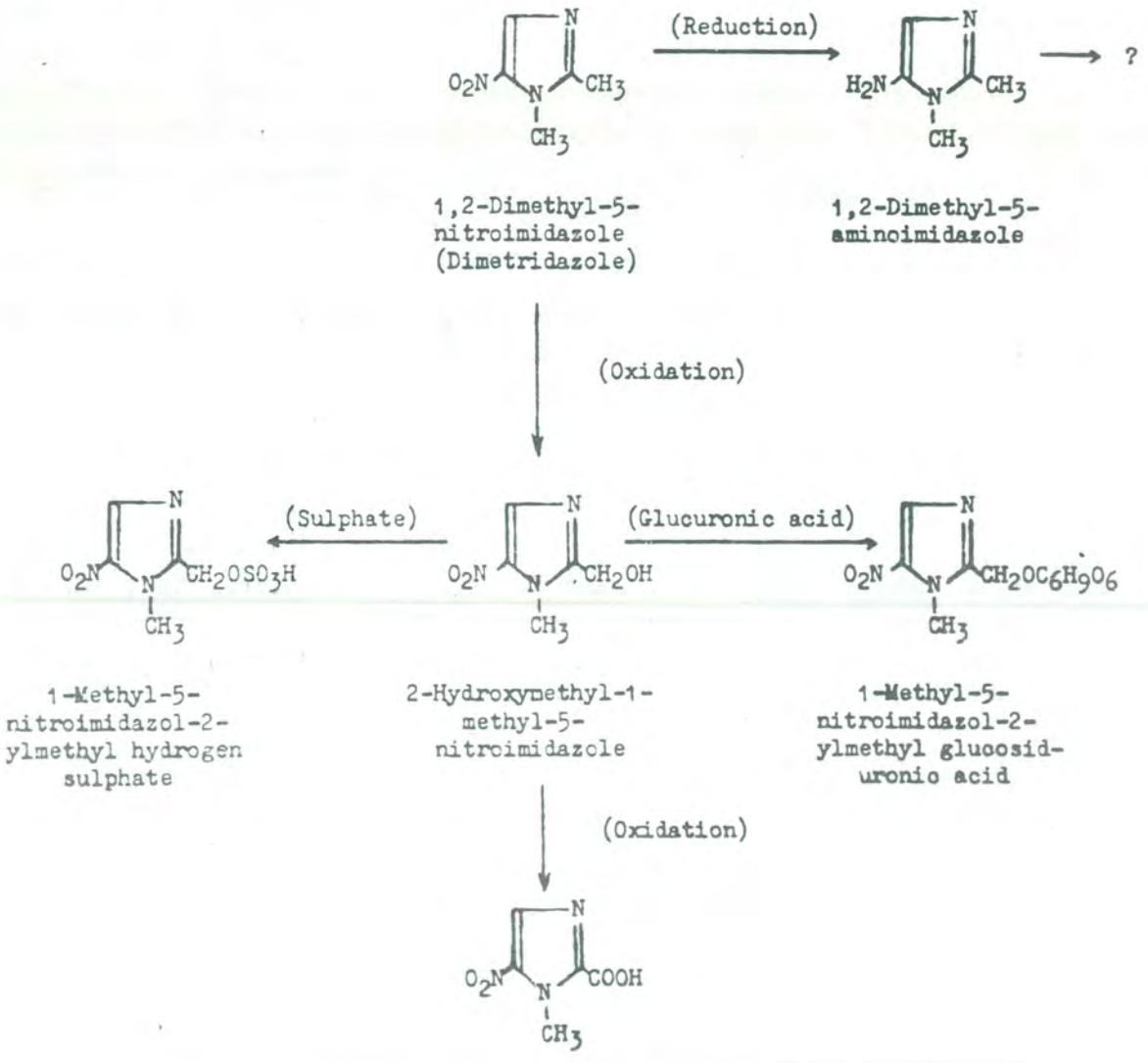
Following a single dose of 100 mg/kg or 300 mg/kg dimetridazole to turkeys, 66.1% and 63% of the dose could be recovered from urine and fecal extracts as determined with polarographic and colorimetric methods (methods that would detect nitro-containing compounds), respectively, over a three-day period. (Law, et al., 1962)

Turkeys were administered a single dose of dimetridazole, labeled as noted above, at a level of 32 mg/kg or unlabeled drug at 100 mg/kg or 300 mg/kg. Examination by ultraviolet light of a paper chromatogram of urine collected at 24 hours post dosing revealed six spots. In addition, a seventh spot was found with autoradiography in a urine sample from the birds treated with labeled drug. Four of the metabolites, comprising a total of 82.8% of the excreted dose, were identified by comparison to standards. Another metabolite, representing 8.8% of the excreted dose, was identified by color reactions as a conjugated glucuronide of a nitroimidazole, presumably HMMNI. The remaining two metabolites, comprising 10.9% of the excreted drug, were shown to be non-nitro containing compounds. One of these two compounds did not absorb uv light and was presumed to be a ring-degraded metabolite, because absorbing uv light is characteristic of ring-intact imidazoles. The metabolites and the percentage of the dose excreted in the urine that they represent are summarized in Table XIII. Most of the excreted drug, therefore, is metabolized via a pathway involving oxidation at the 2-methyl group. The proposed pathway for the metabolism of dimetridazole in turkeys is given in Figure 1. (Law, et al., 1962)

Table XIII. Metabolites Detected in Urine of Treated Turkeys and Their Percentages

Metabolite	% of dose excreted
dimetridazole	3.2
2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI)	9.4
1-methyl-5-nitroimidazole-2-carboxylic acid (MNICA)	25.8
HMMNI sulfate	44.4
HMMNI glucuronide	8.8
unidentified non-nitro containing compound (uv sens)	6.2
unidentified non-nitro containing compound	4.7

Figure 1. Metabolism of Dimetridazole in Turkeys



Swine

Dimetridazole, labeled with 14C in the N-methyl group, was administered in a single oral dose to four pigs at a rate of 19-37 mg/kg. The pigs were maintained in enclosed cages permitting the collection of all labeled products. Mean recovery of radioactivity from the animals up to seven days post medication was determined to be 76.2%, broken down as follows: urine, 39.2%; feces, 33.1%; exhaled air, 3.9%. Chromatographic examination of the urine from dosed pigs collected during the first eight hours after dosing revealed the presence of parent dimetridazole at 0.2% of the urinary activity, HMMNI at 0.7% of the urinary activity and MNICA at 18.7% of the urinary activity. Conjugation of metabolites in swine was not observed to be a major pathway. Much of the radioactivity in urine was found to be associated with an abundance of isotopically labeled compounds, presumed to include purine and pyrimidine bases, proteins, fatty acids, choline, and lower molecular weight compounds such as amino acids and other simple naturally occurring molecules. (Mulcock and Unsworth, 1973a and 1973b)

Dimetridazole, labeled with 14C in the N-methyl group, was administered orally to a pig in a single dose of 29.8 mg/kg. The pig was sacrificed 6 hours after dosing. Samples of tissues were examined by several techniques, including paper and thin-layer chromatography, electrophoresis, and automatic amino acid analysis, for isotopically labeled products. The amounts of dimetridazole, HMMNI and MNICA in tissues are given in Table XIV. (Mulcock and Unsworth, 1973a)

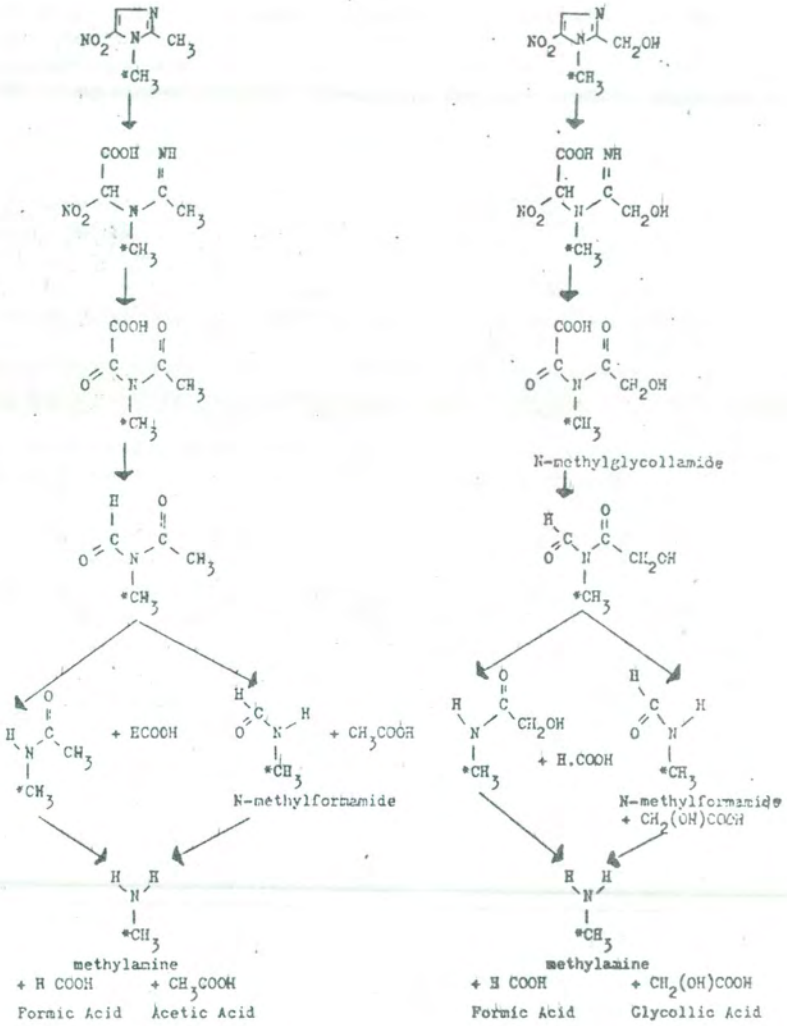
Table XIV. Tissue Residues of Metabolites in Swine Treated with 14C-Dimetridazole (6 hours post dosing)

	Amount of Compound Present					
	As % of Total 14C in specified tissue			In ppm		
Compound	Muscle	Kidney	Liver	Muscle	Kidney	Liver
dimetridazole	0.5	0.5	0.07	0.04	0.18	0.01
HMMNI	40.2	25.7	0.5	3.56	10.31	0.09
MNICA	13.8	3.6	ND	1.33	1.55	ND
Total	54.5	29.8	0.57	4.93	12.04	0.10

In a similar experiment, seventeen hours after dosing a pig with labeled dimetridazole at 16.6 mg/kg the only metabolite detectable was HMMNI in muscle. This metabolite represented about 10% of the tissue radioactivity or 0.04 ppm. (Mulcock and Unsworth, 1973a)

Based on the above work, the metabolism of dimetridazole in swine is believed to include: oxidation at the 2-methyl group to give the hydroxymethyl and carboxylic acid metabolites (see Figure 1); reduction at the 5-nitro group to give the 5-amino compound, which would undergo rapid degradation; and fission of the nitroimidazole ring (Figure 2). (Muggleton and Unsworth, 1974)

Figure 2. Metabolism of Dimetridazole via Fission of the Nitroimidazole Ring



Rats

Dimetridazole, labeled with 14C in the N-methyl group, was administered in a single oral dose to Sprague-Dawley rats at a rate of 25 mg/kg. The results of this study indicate qualitative similarity with those of the study in swine. That is, evidence has been provided for metabolism via oxidation at the 2-methyl group and degradation of the nitroimidazole ring. (Heijbroek, 1976)

METHODS OF RESIDUE ANALYSIS

The early methods of analysis for residues of dimetridazole in tissues of swine and turkeys relied on polarography. These assays had a level of sensitivity in the range of 0.05 to 0.1 ppm. The methods were designed for parent dimetridazole but would likely determine any nitroimidazoles that were extracted. Thus, although HMMNI would likely be measured by those early polarographic methods, the amount of HMMNI extracted would probably be low because of its water solubility. (Kane, 1961; Parnell, 1973)

A gas chromatographic procedure was also developed for parent dimetridazole in turkey tissues with a sensitivity of 2 ppb. (DHHS, 1986)

The finding that HMMNI was a major metabolite both in turkey and swine led to the development of assays that could measure the hydroxy metabolite. Improved assay methodology involving differential pulse polarography brought the limit of detection down to 1 to 2 ppb in swine tissue. The methods probably measure all nitroimidazole residues present in the extract. (Craine, et al., 1974; Ohst, 1987)

Recently, a HPLC procedure with electrochemical detection has been developed. The method has been applied to the simultaneous determination of both dimetridazole and HMMNI in swine tissue to concentrations of about 0.5 ppb. (Sved and Carignan, 1987)

APPRAISAL

The use of dimetridazole at permitted concentrations in the feed and drinking water of poultry and swine produces residues that deplete below detectable levels at 2 to 3 days postdosing. The most sensitive assays used in the depletion studies monitor for dimetridazole and/or HMMNI in the 0.5 to 2 ppb range.

The radiotracer work that was done in swine, however, shows that the total residue of dimetridazole in swine at 7 days postdosing ranges from 320 ppb in muscle to 910 ppb in liver (Table II). Unfortunately, because all residue values reported for dimetridazole or HMMNI are below the limit of detection at times beyond 2 days, it is impossible to establish any relationship between the total residue and a compound (or compounds) measured with a chemical assay.

The metabolism work that has been done supports the proposed pathways. In particular, oxidation at the 2-methyl group is the main route to ring intact metabolites and ring scission leads to fragmentation. It is possible that reduction of the 5-nitro group takes place, which in turn would lead to decomposition of the ring as well. Furthermore, it is likely that some radioactivity becomes incorporated into natural components via fragments from the ring scission of either dimetridazole or of the 2-methyl-oxidized products.

Although not discussed in the reports provided on dimetridazole, it is possible, based on work done for other nitroimidazoles, metronidazole, for example, that acetamide, a known carcinogen (IARC, 1974), can result from the fragmentation of dimetridazole. Moreover, the possibility of the formation of "bound" residues, residues that result from the reaction of a reactive metabolite or metabolic intermediate with natural tissue components, such as protein or nucleic acid, must be considered in view of the work done for ronidazole. At this time the total residue of dimetridazole in tissues of poultry and swine has not been fully characterized.

REFERENCES

Condren, H.B., Davies, R.E., Deyoe, C.W., Zavala, M.A., Creger, C.R. and Couch, J.R. (1963). Studies on the effect of 1,2-dimethyl-5-nitroimidazole on growth and reproduction in turkeys and its residual concentration in tissue. Poultry Science, 42, 585-594.

Craine, E.M. and Anderson, M.D. (1973a). Nitroimidazole residues in tissues of swine medicated with dimetridazole given in drinking water. Report EMC 73:32 from Hess and Clark, Ashland, Ohio. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Craine, E.M. and Anderson, M.D. (1973b). Nitroimidazole residues in tissues of swine fed a protein supplement containing 0.24% dimetridazole. Report EMC 73:50 from Hess and Clark, Ashland, Ohio. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Craine, E.M. and Anderson, M.D. (1973c). Nitroimidazole residues in tissues of swine medicated with 0.0125% dimetridazole in the feed. Report EMC 73:48 from Hess and Clark, Ashland, Ohio. Submitted to FAO by Rhone-Poulenc Sante, Paris

Craine, E.M., Parnell, M.J., and Stone, L.R. (1974). A method for analysis of swine tissue for the primary metabolite of dimetridazole at the 2 ppb level. J. Agric. Food Chem., 22, 877-881.

Department of Health and Human Services (1986). Dimetridazole; Opportunity for Hearing; Notice. Federal Register, 51, 45244-45262.

Heijbroek, W.M.H. (1976). Dimetridazole excretion and metabolism studies in rats. Report RES/2498 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

International Agency for Research on Cancer (1974). Some antithyroid and related substances, nitrofurans and industrial chemicals. WHO, Lyon, 7, 197-202.

Kane, P.O. (1961). Polarographic methods for the determination of two anti-protozoal nitroimidazole derivatives in materials of biological and non-biological origin. J. Polarogr. Soc., 8, 73-78.

Law, G.L., Mansfield, G.P., Muggleton, D.F. and Parnell, E.W. (1962). Dimetridazole (8595 R.P.): absorption, excretion and metabolism in turkeys. Report A.P.C.D./131 Biochem/246 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Law, G.L., Mansfield, G.P., Muggleton, D.F. and Parnell, E.W. (1963). Dimetridazole: absorption, excretion and metabolism in turkeys. Nature, 197, 1024-1025.

Muggleton, D.F. (1963). Dimetridazole residues in chicken tissues after oral administration in prophylactic and therapeutic amounts. Report A.P.C.D./136 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Muggleton, D.F. and Unsworth, J.B. (1974). Dimetridazole studies with the 14C-labelled compound in turkeys and pigs; a review. Report RG/2074 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Mulcock, P.A. and Unsworth, J.B. (1973a). Dimetridazole metabolism studies in pigs. Report RG/1612 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Mulcock, P.A. and Unsworth, J.B. (1973b). Dimetridazole excretion studies in pigs. Report RG/1613 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Ohst, E. (1987). Development of a method for detecting nitroimidazole residues in pig muscle. Report No. 1186. Submitted to FAO by BASF Aktiengesellschaft, Ludwigshafen.

Parnell, M.J. (1973). Determination of dimetridazole in pig tissue I. Method. Pestic. Sci., 4, 643-646.

Sved, S. and Carignan, G. (1987). The kinetics of elimination of dimetridazole and its hydroxylated metabolite in food animals. Presented at: AOAC Spring Workshop, Ottawa, Canada.

Unsworth, J.B. (1972). Dimetridazole absorption and excretion studies in the pig. Report RG/1502 from May and Baker Ltd., Dagenham, Essex. Submitted to FAO by Rhone-Poulenc Sante, Paris.

Windholz, M. ed. (1983). The Merck Index 10th Edition. Rahway, N.J., Merck and Co.

DIMINAZENE ACETURATE

IDENTITY

Chemical name: 4,4'-(diazoamino)dibenzamidine diaceturate

1,3-bis(p-amidinophenyl)triazine bis(N-acetyl-glycinate)

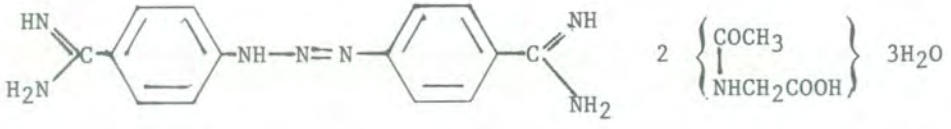
1,3-bis[4-guanylphenyl]triazine diaceturate

4,4'diamidinodiazminobenzene diaceturate

p,p'-diguanyldiazoaminobenzene diaceturate

Synonyms: Azidin; Ganasag; Berenil

Structural formula:



Molecular formula: $C_{22}H_{29}N_9O_6$

Molecular weight: 515.54

OTHER INFORMATION ON IDENTIY AND PROPERTIES

Pure active ingredient:

Appearance: Yellow solid

Melting Point: Decomposes at 217°C

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Diminazene is an antitrypansmal agent. Although it is principally used for the therapeutic treatment of trypanosomiasis in cattle, efficacy has been demonstrated in rabbits (Gilbert and Newton, 1982; Gilbert, 1983). In addition, pharmacokinetic evaluations have also conducted in sheep (Aliu and Odegaard, 1985), goats (Aliu, et al., 1984) and rats (Fussganger and Bauer, 1958; Raether et al., 1972). Activity has been demonstrated against Trypanosoma congolense, T. brucci, T. gambiense, T. rhodesiense, T. lewsisi, and Babesia canis (Fussganger and Bauer, 1958).

Dosage

The recommended therapeutic dose of diminazene aceturate is 3.5 mg/kg administered intramuscularly or 2.0 mg/kg administered intravenously as a sterile suspension.

Pharmacokinetics

The pharmacokinetics of diminazene have been investigated in the laboratory and target animal species. In general, the peak blood levels (Cmax) were reached within 1 hour after dosing (Aliu et al., 1985,; Gilbert and Newton, 1982; Gilbert, 1983; Kellner, et al., 1985; Raether, et al., 1972; Raether, et al., 1974; Klatt and Hajdu, 1971; and Aliu and Odegaard, 1985). The only exception was the dog, in which peak levels were not reached until 3 hours after dosing (Fussganger and Bauer, 1958). The peak blood levels were 1.1 ppm in the rabbit (Gilbert and Newton, 1982; Gilbert, 1983), 13 ppm in the rat (Raether et al., 1972), 4.6 to 6 ppm in cattle (Kellner et al., 1985; Klatt and Hajdu, 1971) and 3.9 to 6.7 in goats (Aliu et al., 1985) and sheep (Aliu and Odegaard, 1985).

The elimination from the blood was generally biphasic, with a half-life of approximately 1.3 hours for the initial phase and up to 188 hours for the terminal phase. However, the studies by Aliu and Odegaard (1985) indicated that the sheep displayed a triphasic elimination curve which is indicative of a three-compartment model. In cattle, the drug was primarily excreted in the urine (Kellner et al., 1985). More than seven times as much radioactivity was found in the urine than in the feces in this species. By day 20 after dosing, more than 80% of the dose had been eliminated.

METHODS OF RESIDUE ANALYSIS

Several techniques have been reported for the determination of diminazene in plasma, These methods include paper and thin-layer chromatography, (Clark, 1969), colorimetry (Raether, et al., 1972) and high performance liquid chromatography (Aliu and Odegaard, 1983). Except for the method of Aliu and Odegaard (1983), most assays were either non-specific, insensitive to submicrogram levels, or they involved tedious and protracted sample preparation steps.

Determinations of diminazene in milk have been conducted using a colorimetric method (Klatt and Hajdu, 1971) and a high performance liquid chromatographic (HPLC) method (Aliu and Odegaard, 1985). The colorimetric method reported a sensitivity of 0.07 ppm in milk using a wavelength of 540 nm. The HPLC method used paired-ion extraction and an internal standard which produced a sensitivity of 0.05 ppm in plasma.

The investigations of diminazene residues in tissues were generally conducted using either radio-tracer analysis (Gilbert, 1983) or colorimetry (Klatt and Hajdu, 1976).

METABOLISM AND RESIDUES STUDIES

Metabolism Studies

Investigations using radiolabeled diminazene administered intramuscularly to cattle at 3.5 mg/kg have shown that the compound is eliminated in the urine and feces according to a biphasic process (Kellner, et al., 1985). The terminal half-lives were 173 hours for the urine and 207 hours for the feces. Two metabolites, in addition to the parent compound, were found in the urine of one of the animals by thin layer chromatography. They were determined to be: 1) p-aminobenzamidine (22% of the radioactivity) and 2) p-amino-benzamide (4% of the radioactivity).

Residue Studies

Studies in rabbits given 3.5 mg/kg intramuscularly showed that liver contained the most residues after 7 days withdrawal. Those residues represented 35 to 50% of the dose (about 40 ppm). The residues in the kidneys after 7 days were approximately 3.3 ppm (Gilbert, 1985).

The residue pattern in the edible tissues of cattle 7 days after dosing with 3.5 mg/kg diminazene indicated that liver contained the highest concentrations of residues (75 ppm) followed by the kidneys at 55 ppm. The concentrations in the other tissues were much lower with the lowest values being found in the fatty tissue and skeletal muscle (Kellner et al., 1985). The pattern was similar 20 days after dosing but the concentrations were lower. The results of this study have been tabulated in Table I. A similar study was conducted in cattle using an 8 mg/kg dose (Klatt and Hajdu, 1976). The patterns found in that study were basically the same as those noted by Kellner et al.

TABLE I. Diminazene residues in calves (ppm Equivalents)

Tissue	7 days	20 days
Liver	75.49	24.42
Kidney	54.67	12.12
Spleen	2.51	1.00
Muscle	0.52	0.26
Injection Site	0.69	0.64
Fat	0.20	<0.18

The excretion of diminazene in cow's milk has been investigated after administration of a 3.5 mg/kg dose (Klatt and Hajdu, 1971). The highest milk levels were found 6 hours after dosing (0.2 - 0.5 ppm). The levels were below the limit of detection (0.07 ppm) 48 hours after dosing.

Peak levels (1.68 ppm) of diminazene were found in the milk of goats 4 hours after the intravenous administration of 2 mg/kg (Aliu et al., 1985). In the same study using a 3.5 mg/kg intramuscular dose, only trace amounts (0.05 ppm) of diminazene could be detected after 72 hours.

Recommended Withdrawal Times

Chronic (9-month) toxicity testing of the oral administration of diminazene in rats and dogs indicated a no-effect level of 20 mg/kg body weight (Baeder, et al., 1975; Scholz and Brunk, 1969). Allowing a safety factor of 100, the acceptable daily intake value (ADI) for a 60 kg human was calculated to be 12 mg. Based on this figure, the Federal Republic of Germany has set a withdrawal period of 20 days for cattle and sheep. By 20 days, the levels are lower than the ADI value.

Pharmacokinetic studies for diminazene in sheep were conducted by Aliu and Odegaard (1985) in Norway. Using a three-compartment open model the authors calculated the clearance of diminazene. Based on the data they obtained, a preslaughter withdrawal time of 14-26 days in sheep was recommended. However, the paper indicated that since liver contains the highest concentrations of the residues, the withdrawal period could be more accurately estimated from the time drug concentrations in the liver.

APPRAISAL

The intramuscular use of diminazene in food-producing animals produce significant residues in the liver and kidney tissues. However, the levels and persistence of these residues were significantly less than those found for a similar antitrypansmal agent, isometamidium.

With the chronic toxicity testing of the oral administration of diminazene, the acceptable daily intake (ADI) for man at 12 mg could be calculated. This provides for very high safe concentration levels and therefore minimal human food safety concerns. Therefore, the recommended withdrawal period prior to slaughter of 20 days for cattle and sheep as well as the 3 day milk discard period, should be adequate.

REFERENCES

Aliu, Y.O. and Odegaard, S. (1983). Paired-ion Extraction and High-performance Liquid Chromatographic Determination of Diminazene in Plasma. J. of Chrom. 276 218-223.

Aliu, Y.O., Odegaard, S. and Sogen, S. (1984). Diminazene/Berenil: Bioavailability and Disposition in Dairy Goats. Acta Vet. Scandinavica, 55 4560.

Aliu, Y.O. and Odegaard, S. (1985). Pharmacokinetics in Sheep. J. of Pharm. and Biopharm, 13 (2) 173-184.

Baeder, Shulz, and Kramer. (1975). Eine Vertraglichkeitsprufung von Berenil-diazeturat an Wistar-Ratten uber 3, 6 und 9 Montae bei oraler Verbreichung im taglichen Futter und mit der Sonde. Hoechst Toxicology Research Report 28.11.1975.

Clark, E.G.C., (Editor) (1969). Isolation and Identification of Drugs, Vol 1, Pharmaceutical Press, London, 312.

Fussganger, R. and Bauer, F.(1958). Berenil ein neues Chemotherapeuticum in der Veterinar -medizin. Medizin und Chemie, 6 504-531.

Gilbert, R.J. and Newton, B.A. (1982). Pharmacokinetics and Efficacy of the Trypanocide Diminazene Aceturate (Berenil) in Rabbits. Vet. Rec., 111 397.

Gilbert, R.J. (1983). Studies in Rabbits on the Disposition and Trypanocidal Activity of the Anti-trypanosomal Drug, Diminazene Aceturate (Berenil). Br. J. Pharmac., 80 133-139.

Kellner, H. M., Eckert, H.G. and Volz, M.H. (1985). Studies in Cattle on the Disposition of the Anti-trypanosomal Drug Diminazene Diaceturate (Berenil). Trop. Med. Parasit. 36 199-204.

Klatt, P. and Hajdu, P. (1971). Blutspiegeluntersuchungen und Milchausscheidung mit Berenil ad us.vet. an Rindern. Hoechst Research Report, October 1971.

Klatt, P. and Hajdu, P. (1976). Ruckstandsbestimmungen in Organen von Rindern nach intramuskularerer Applikation von Berenil. Hoeschst Research Report 5.3.76.

Raether, W., Hajdu, P., Seidenath, H. and Damm, D. (1972). Pharmacokinetictsche und chemoprophylaktische Untersuchungen mit Berenil an Wistar-Ratten (Trypanosoma rhoddesiense). Z. Tropenmed. Parasit. 23 418-427.

Raether, W., Hajdu, P., Seidenath, H. and Damm, D. (1974). Pharmakokinetische und chemoprophylaktische Untersuchungen mit Berenil an Maken (Trypnaosoma rhodesiene -Infection). Z. Tropenmed. Parasit. 25 42-48.

Scholz and Brunk. (1969). Chronische orale Toxizitatsprufung von Berenil-diaceturat 9-Monate-Versuch an Hunden. Hoechst Research Report 21.1.1969.

IPRONIDAZOLE

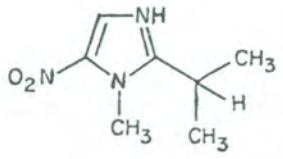
IDENTITY

Chemical name: 2-isopropyl-1-methyl-5-nitroimidazole

1-methyl-2-(1-methylethyl)-5-nitro-1H-imidazole

Synonyms: Ipropran

Structural formula:



Molecular formula: $C_7H_{11}N_3O_2$

Molecular weight: 169.18

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: white plates

Melting point: 60°C

(Windholz, 1983)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Ipronidazole is used in turkeys (1) for the prevention and treatment of blackhead (histomoniasis) and (2) for increased rate of weight gain and improved feed efficiency.

The use of the drug for controlling and treating swine dysentery has also been reported. (Messersmith, et al., 1973)

Dosages

Ipronidazole is administered through the feed at levels of approximately 0.00625% (prevention) to 0.025% (treatment). In addition, the drug may be given via the drinking water at 0.0125% to turkeys already receiving 0.00625% in the feed for treatment purposes.

Continuous feeding of ipronidazole at a concentration of 100 g/ton in feed has been shown to prevent swine dysentery. Administration of ipronidazole at 200 to 800 mg/gal of drinking water for seven days was claimed to be effective in treating swine dysentery. (Messersmith, et al., 1973)

RADIOLABELED RESIDUE DEPLETION STUDIES

Turkeys

Two 14-week-old turkeys were pretreated from 1 day of age to 14 weeks of age with a medicated feed containing 0.00625% ipronidazole. A single oral capsule of 20 mg of ipronidazole-2-14C (equivalent to 0.00625% in the feed) was administered to each turkey at 14 weeks of age. The turkeys were sacrificed at 5 days of withdrawal. The average concentration of total radioactivity (ppb) in the edible tissues of treated birds were: breast muscle, 64.85; leg and wing muscle, 71.21; liver 285.40; kidney, 257.72; fat, 24.81; and skin/fat, 92.18. (Laurencot, et al., 1977)

Bioavailability of Turkey Tissue Residue in Rat

The liver or the muscle tissue from the birds of the study described above was lyophilized and pelleted and fed to rats. The liver tissue had a concentration of total residue expressed as ipronidazole equivalents of 0.96 ppm and the muscle, 0.22 ppm. Rat urine, bile, and feces were collected for 24 before treatment, during the 24-hour treatment period on turkey tissue, and for 24 hours after treatment. The rats were killed 24 hours after treatment and the carcass, liver, GI tract, and GI tract contents were taken for assay of total radioactivity.

For rats treated with lyophilized and pelleted turkey liver tissue, an average of 56% of the radioactivity was found in the feces and 0.36% in the contents of the GI tract, for a total of 56.36% as non-absorbed radioactivity. An average of 2.77% was found in the bile, 3.11% in the carcass, 0.25% in the GI tract tissue, 0.42% in the liver and 42.8% in the urine, for a total of 49.35% as absorbed radioactivity from liver tissue. Total recovery averaged 105.71%. (Laurencot, et al., 1977)

For rats treated with lyophilized and pelleted muscle tissue, an average of 23.3% of the radioactivity was found in the feces and 0.38% in the contents of the GI tract, for a total of 23.68% as non-absorbed radioactivity. An average of 5.96% was found in the bile, 8.23% in the carcass, 0.51% in the GI tract tissue, 0.73% in the liver and 52.35% in the urine, for a total of 67.78% as absorbed radioactivity from muscle tissue. Total recovery averaged 91.46%. (Laurencot, et al., 1977)

Swine

A 4-week-old, 7 kg male pig was treated with a single oral capsule of ipronidazole-2-14C at 43.11 mg (equivalent to 0.0126% in the feed). The pig was sacrificed at 5 days of withdrawal and tissue samples were taken for radioassay by combustion analysis. Total residue concentrations (ppb) in the edible tissues were: muscle, 41.1; liver, 192.7; kidney, 189.5; fat, 20.6; and skin, 36.3. (Laurencot, et al., 1978)

Bioavailability of Swine Tissue Residue in Rat

The liver tissue from the pig of the study described above was lyophilized and fed to 4 rats. The liver tissue had a concentration of total residue expressed as ipronidazole equivalents of 0.62 ppm. Over the collection period of the experiment, an average of 45.61% of the radioactivity was found in the feces and 0.09% in the GI tract contents, for a total of 45.70% as non-absorbed radioactivity. An average of 1.72% was found in the bile, 0.13% in the GI tract tissue, 0.52% in the liver and 42.54% in the urine, for a total of 44.91% as absorbed radioactivity from liver tissue. Total recovery averaged 90.61%. (MacDonald, 1977a)

RESIDUE DEPLETION STUDIES

Turkeys

Nine-week-old turkeys were placed on feed medicated with ipronidazole at the 0.00625% level for 12 weeks. Birds were sacrificed after a withdrawal period of 0, 1, 2, 3, 4, 5 or 6 days. The concentration of ipronidazole and the hydroxy metabolite, 1-methyl-2-

(2'-hydroxyisopropyl)-5-nitroimidazole (HIP), was determined in the tissues of ten birds at each withdrawal period. Assays were done using gas chromatography (GC) with electron capture detection specific for each compound to 2 ppb. No ipronidazole was detectable in tissues of the treated turkeys. The concentrations of HIP (ppb) are given in Table I. No HIP was detected in any edible tissue beyond 4 days post dosing. (Kaykaty, 1969; MacDonald, et al., 1971)

Table I. Concentration of HIP in Turkeys Dosed with 0.00625% Ipronidazole in the Feed (ppb)

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	64.2	ND	ND	90
1	21.5	ND	ND	8.5
2	1.2	ND	ND	3.6
3	ND	ND	ND	2.0
4	ND	ND	ND	ND

ND = not detected

Male turkeys which were 24-weeks old received ipronidazole in the drinking water at a dose of 0.0125% for seven days. Birds were sacrificed at zero withdrawal and at days 1 through 9 of withdrawal. The concentration of ipronidazole and HIP was determined in the tissues of five birds at each withdrawal time. The concentrations of ipronidazole and HIP (ppb) are shown in Table II. No residues of ipronidazole were observed beyond the 1-day withdrawal and none of HIP beyond the fourth day of withdrawal. Analyses were done with the GC procedure sensitive to 2 ppb. (Fellig, et al., 1971)

Table II. Concentrations of Ipronidazole and HIP in Turkeys Treated With 0.0125% Ipronidazole in the Drinking Water (ppb)

Ipronidazole

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	0.9	ND	ND	52.0
1	ND	ND	ND	0.8

HIP

Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	24.5	ND	ND	35.5
1	8.0	ND	ND	22.1
2	0.6	ND	ND	5.5
3	0.4	ND	ND	4.2
4	ND	ND	ND	0.4

ND = not detected

Male turkeys, 24 weeks of age, received ipronidazole at a dose of 0.025% (therapeutic level) in the feed for seven days. Birds were sacrificed at zero withdrawal and at days 1 through 9 of withdrawal. The concentration of ipronidazole and HIP was determined in the tissues of five birds at each withdrawal time. The concentrations of ipronidazole and HIP (ppb) are shown in Table III. No residues of ipronidazole were observed beyond the 1-day withdrawal and none of HIP beyond the second day of withdrawal. Analyses were done with the GC procedure sensitive to 2 ppb. (Fellig, et al., 1972a)

Table III. Concentrations of Ipronidazole and HIP in Turkeys Treated With 0.025% Ipronidazole in the Feed (ppb)

	Ipronidazole			
Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	1.4	ND	ND	115.0
1	ND	ND	ND	0.5

	HIP			
Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	106.8	ND	ND	66.2
1	17.7	ND	ND	19.9
2	ND	ND	ND	0.6

ND = not detected

Fifty-four 18-week-old turkeys, mixed as to sex, received ipronidazole at 0.00625% in the feed for four weeks, followed by drug at 0.0625% (10 times the recommended use level) for two days. Birds were sacrificed at zero withdrawal and at days 1 through 6 of withdrawal. The concentration of ipronidazole and HIP was determined in the tissues of five birds at each withdrawal time. The concentrations of ipronidazole and HIP (ppb) are shown in Table IV. No residues of ipronidazole were observed beyond the 1-day withdrawal and none of HIP beyond the third day of withdrawal. Analyses were done with the GC procedure sensitive to 2 ppb. (Fellig, et al., 1972b)

Table IV. Concentrations of Ipronidazole and HIP in Turkeys Treated With Ipronidazole at 0.0625% in the Feed (ppb)

	Ipronidazole			
Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	10.2	ND	ND	8.2
1	ND	ND	ND	1.6

	HIP			
Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	1185.5	ND	ND	957.3
1	113.0	ND	ND	152.4
2	0.8	ND	ND	2.2
3	ND	ND	ND	0.8

ND = not detected

Fifty 18-week-old male and female turkeys were administered 0.00625% ipronidazole via the feed for seven weeks and 0.0125% ipronidazole via the drinking water for the last seven days of the seven-week period. Birds were sacrificed at zero withdrawal and at days 1 through 7 of withdrawal. The concentration of ipronidazole and HIP was determined in the tissues of five birds at each withdrawal period. The concentrations of ipronidazole and HIP (ppb) are given in Table V. No residues of ipronidazole were observed beyond 1 day of withdrawal and none of HIP beyond the second day of withdrawal. Analyses were done with the GC procedure sensitive to 2 ppb. (Fellig, et al., 1972c)

Table V. Concentrations of Ipronidazole and HIP in Turkeys Treated With Ipronidazole in the Feed and Drinking Water (ppb)

	Ipronidazole			
Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	0.6	ND	ND	96.9
1	ND	ND	ND	0.3

	HIP			
Withdrawal Time (days)	Muscle	Liver	Kidney	Skin/Fat
0	141.5	ND	ND	172.4
1	0.9	ND	ND	3.3
2	ND	ND	ND	0.6

ND = not detected

Swine

Eighteen 5-week-old pigs received 200 g/ton ipronidazole through the feed for 6 weeks. Three animals were sacrificed at 0, 3, 4, 5, 6 and 7 days of withdrawal. Samples of muscle, liver, kidney and fat were collected and assayed for ipronidazole and HIP with a GC procedure sensitive to 50 ppb for each compound. No residues of ipronidazole were found in any of the edible tissues. Residues of HIP averaged 327 ppb in muscle and 47 ppb in fat at 0 withdrawal; otherwise, no residues of HIP were observed in any edible tissue at any withdrawal time. (MacDonald, 1969)

Eighteen 5-week-old pigs received 100 g/ton ipronidazole through the feed for 6 weeks. Three animals were sacrificed at 0, 2, 3, 4, 5 and 6 days of withdrawal. Samples of muscle, liver, kidney and skin/fat were collected and assayed for ipronidazole and HIP with a GC method sensitive to 2 ppb for each compound. Residues of ipronidazole averaged 1.6 ppb in skin/fat and 0.5 ppb in muscle at 0 withdrawal; otherwise, none was detected in any tissue sample at any withdrawal time. Residues of HIP averaged 77 ppb in skin/fat and 167.5 ppb in muscle at 0 withdrawal; otherwise, none was detected in any sample at any withdrawal time. (Kaykaty, 1970)

Twenty 7-week-old pigs, equally mixed as to sex, were placed on feed containing 100 g/ton ipronidazole for 14 weeks. Three animals were sacrificed on each of days 0 through 5 of withdrawal. Samples of liver, kidney, internal fat, subcutaneous fat, thigh muscle and loin muscle were collected for analysis with the GC procedure having a sensitivity of 2 ppb. At 0 withdrawal, residues of ipronidazole averaged 2.8 ppb in loin muscle, 1.4 ppb in thigh muscle, 1.9 ppb in subcutaneous fat and 3.4 ppb in internal fat. At zero withdrawal, residues of HIP were 43.2 ppb in loin muscle, 29.8 ppb in thigh muscle, 15.5 ppb in subcutaneous fat and 7.1 ppb in internal fat. No other samples at any withdrawal time contained detectable residues of ipronidazole or HIP. (Fellig, et al., 1973a) (Fellig, et al., 1975)

Twenty-three pigs, mixed as to sex and averaging 77.6 kg, were treated with ipronidazole through an oral aqueous solution at 44 mg/kg body weight for 7 consecutive days (equivalent to 7 to 15 times the dose expected via 100 g/ton in the feed). Three animals were sacrificed on each of withdrawal days 0 through 6 and two were killed on day 7 of withdrawal. Samples of liver, kidney, skin/fat, internal fat, thigh muscle and loin muscle were taken for analysis with the GC procedure sensitive to 2 ppb. The results of the analyses are shown in Table VI. No residues of ipronidazole or HIP were found in any tissues beyond 5 days of withdrawal. (Fellig, et al., 1973b) (Fellig, et al., 1975)

Table VI. Concentrations of Ipronidazole and HIP in Swine Treated With Ipronidazole Via An Oral Aqueous Solution (ppb)

Ipronidazole

Withdrawal Time (days)	Thigh Muscle	Loin Muscle	Liver	Kidney	Skin Fat	Int. Fat
0	448.1	433.4	ND	5.4	545.9	843.5
1	98.9	69.0	ND	1.7	20.3	55.1
2	9.3	5.6	ND	0.9	10.3	32.0
3	1.2	ND	ND	ND	2.4	3.6
4	7.5	ND	ND	ND	2.4	1.7
5	ND	ND	ND	ND	ND	2.0

HIP

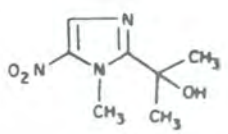
Withdrawal Time (days)	Thigh Muscle	Loin Muscle	Liver	Kidney	Skin Fat	Int. Fat
0	4913.3	3296.0	ND	2.1	934.4	2064.0
1	72.0	196.0	ND	ND	64.6	74.4
2	4.4	11.4	ND	ND	19.1	5.6
3	1.1	ND	ND	ND	1.3	ND
4	0.5	ND	ND	ND	1.2	ND

ND = not detected

METABOLISM STUDIES

Turkeys

The only two drug-related compounds identified in the tissues of turkeys administered ipronidazole are parent ipronidazole and the hydroxy-isopropyl metabolite (1-methyl-2-(2'-hydroxyisopropyl)-5-nitroimidazole, HIP) formed by oxidation of the alkyl group at the 2-position of ipronidazole. The structure of HIP, shown below, was reported to be deduced from infrared spectroscopy, nuclear magnetic resonance, ultraviolet spectroscopy and mass spectrometry, and confirmed by comparison to a synthesized standard. Unchanged drug and HIP account for about 40% of the excreted dose. The remaining metabolites are highly water-soluble and cannot be extracted into organic solvents before or after enzymic hydrolysis. (Fellig, et al., 1969) (Weiss, et al., 1981)



1-methyl-2-(2'-hydroxyisopropyl)-5-nitroimidazole, HIP

Swine

A 4-week-old, 7 kg male pig was treated with a single oral dose of ipronidazole-2-14C at 43.11 mg (equivalent to 0.0126% in the feed). Five days following treatment 97.9% of the radioactivity had been recovered in the urine, feces and tissues. A breakdown of the total residue in edible tissues by extractability is given in Table VII. Approximately 31 to 71% of the residue in edible tissues is water soluble. Moreover, none of the radioactivity of gut contents, which comprised 53.3% of the total recovered radioactivity, was benzene extractable, indicating that ipronidazole and HIP were not present. Of interest also, approximately 29 to 68% of the residue in edible tissues was unextractable. The unextractable residue may reflect covalent binding (bound residue) or incorporation of fragments from ipronidazole into natural components. No metabolites were identified in this study. (Laurencot, et al., 1978)

Table VII. The Concentration and Distribution of Radioactivity of RO 7-1554-2^{14}C and Its Possible Metabolites in the Benzene Soluble, Water Soluble and Insoluble Residue Fractions of the Edible Tissues of a Four Week Old Male Cross Bred Chester White Swine Five Days after Treatment

	Treated Tissues		Control Tissues	
	µg	ppb	Minimal Detectable ppb	Background Count Error ppb
Fat				
Benzene soluble	0.5473	2.9518	0.0287	0.0197
H_2O soluble	1.4274	7.6989	0.9741	0.6703
Insoluble residue	1.8456	9.9547	0.0480	0.0330
Kidney				
Benzene soluble	0.0020	0.0512	0.1320	0.0906
H_2O soluble	5.1104	129.4420	2.3255	1.5874
Insoluble residue	2.3706	60.0448	0.0750	0.0515
Liver				
Benzene soluble	0.0291	0.1046	0.0486	0.0335
H_2O soluble	38.0718	136.9489	1.0541	0.7244
Insoluble residue	15.4623	55.6198	0.0304	0.0209
Muscle				
Benzene soluble	0.9032	0.4756	0.0508	0.0349
H_2O soluble	24.0375	12.6580	1.6148	1.0956
Insoluble residue	53.1060	27.9652	0.1187	0.0816
Skin				
Benzene soluble	2.3392	2.0934	0.0512	0.0351
H_2O soluble	15.4286	13.8076	2.0257	1.3920
Insoluble residue	22.7530	20.3624	0.1110	0.0763
Blood				
Benzene soluble	0.0180	0.0704	-	-
H_2O soluble	12.1249	47.5487	-	-
Insoluble residue	1.1192	4.3890	-	-

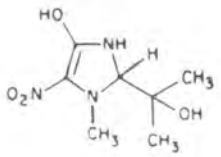
Ipronidazole-2-14C was incubated with a fortified swine liver microsome preparation under aerobic and anaerobic conditions. The benzene soluble metabolites, both before and after glusulase (a preparation of beta-glucuronidase and sulfatase) incubation, were separated by paper chromatography, identified by radiochromatogram scanning, and quantified by

sample oxidation and liquid scintillation counting. The only metabolite found in the benzene extracts of the aerobic incubation was HIP, with 31.2% being free and 3.8% conjugated. Neither ipronidazole nor HIP was found in the benzene extracts of the anaerobic system containing FAD (a nitro-reduction enhancer) either before or after glusulase incubation. (Laurencot, et al., 1975)

Rats

In two separate studies, it was observed that following a single intraperitoneal dose of 20.5 mg/kg ipronidazole-2-14C, the urine of treated rats contained about 30% of the administered radioactivity, the feces, approximately 58%, and expired air, about 3% of $14CO_2$. Only 0.5% of the treatment radioactivity was benzene extractable from the urine and 1.0% benzene extractable from the feces before and after glusulase incubation. Paper and thin layer chromatography of the whole urine, and water and benzene extracts of the feces indicated that neither ipronidazole nor HIP was present. It was concluded that the radioactivity remaining at the origin in the chromatograms indicates the polar nature of the components and that the production of $14CO_2$ demonstrated the opening of the imidazole ring structure. (MacDonald, 1977b)

A novel metabolite was identified in the water soluble extract of feces of bile-cannulated rats. Fasted female rats with stomach cannulae were treated with ipronidazole-2-14C at 20 mg/kg for 4 days. The results show that 71% of the radioactivity in feces (the feces comprise 31% of the total administered activity) was water soluble. By a number of analytical techniques, including nuclear magnetic resonance, infrared spectroscopy, and mass spectrometry, about 34% of the water extractable fecal metabolites was identified as 2,3-dihydro-2-(2'-hydroxyisopropyl)-3-methyl-4-nitro-1H-imidazol-5-ol (see structure below). Approximately 15% of the total dose in cannulated rats was isolated as this water soluble metabolite. (Weiss, et al., 1978) (Weiss, et al., 1981)



2,3-dihydro-2-(2'-hydroxyisopropyl)-3-methyl-
4-nitro-1H-imidazol-5-ol

METHODS OF RESIDUE ANALYSIS

The earliest procedure for the residue analysis of ipronidazole in edible tissues relied on pulse polarography and gas chromatography. The method had a sensitivity of 50 ppb for unchanged ipronidazole and the hydroxy metabolite, HIP. (MacDonald, et al., 1969)

An improved gas chromatographic procedure lowered the level of sensitivity for each of the analytes in edible tissues of treated swine and turkeys to 2 ppb. The initial tissue extraction with benzene is followed by silica gel column clean-up and concentration. The column eluate is divided for individual extraction procedures followed by a gas chromatographic assay using electron capture detection specific for each compound. This method is accepted as the approved regulatory assay by the United States Food and Drug Administration. (MacDonald, et al., 1971) (FDA, 1988)

Subsequently, two procedures relying on mass spectrometric (MS) techniques were put forth to confirm the identity of ipronidazole and HIP in edible tissues of turkeys. In the first method, tissue is processed according to the procedures outlined for the electron capture-GC regulatory assay. Portions of the resulting benzene extracts are then analyzed by GC-MS using selective ion detection. For ipronidazole the m/e 169 molecular ion and the m/e 123 fragment ion are monitored, while for HIP the mass spectrometer is set to collect the m/e 185 molecular ion and the m/e 128 fragment ion. (Garland and Gonzales, 1975)

In the second method, portions of the benzene extract from the tissue work-up are also analyzed using selective ion monitoring. Methane is used both as the GC carrier gas and negative chemical ionization reagent gas. For the determination of ipronidazole and HIP, molecular ions at m/e 169 and 185, respectively, are monitored in the GC effluent. Deuterated analogues of ipronidazole and HIP are added at the 2 ppb level to tissue before

processing as described in the paragraph above to establish the retention times, extraction efficiencies, mass spectral responses and chromatographic peak shapes of both ipronidazole and HIP. (Garland, et al., 1980)

For a variety of reasons, including lack of sufficient recovery data, severe interferences, and inadequacy of single ion monitoring, neither of the above methods was found acceptable

APPRAISAL

The use of ipronidazole at permitted concentrations in the feed and drinking water of turkeys produces residues that deplete below detectable levels at 3 to 4 days post dosing. The assay used in the depletion studies had a sensitivity of 2 ppb for ipronidazole and the hydroxy metabolite, HIP.

Tissues of swine treated with ipronidazole through the feed at the recommended level of 100 g/ton were free of detectable residues at one day of withdrawal. Tissues of swine treated with a therapeutic dose of ipronidazole via an aqueous oral solution were free of residues at 6 days of withdrawal. The assay used in these studies was also sensitive to 2 ppb for ipronidazole and HIP.

Although the residue depletion studies in turkeys show that residues in edible tissues fall below 2 ppb in 3 to 4 days, the radiotracer work demonstrates that at 5 days of withdrawal the total residue ranges from 24.8 ppb in fat to 285.4 ppb in liver and to 257.7 ppb in kidney . Likewise, the total residue in swine at 5 days of withdrawal ranges from 20.6 ppb in fat to 192.7 ppb in liver and to 189.5 ppb in kidney. The residue depletion data taken as a whole are insufficient for establishing a relationship between the total residue and a compound (ipronidazole and/or HIP) measured with a chemical assay.

The metabolism work that has been done supports the proposed pathways that characterize the 5-nitroimidazoles. In particular, oxidation occurs at the 2-alkyl group; reduction can take place in the 5-nitro group; scission of the molecule occurs, with fragmentation of the imidazole ring; and incorporation of the fission products into natural tissue components proceeds to some degree. It is also possible that bound residues form, and this must be taken into account during the safety evaluation.

REFERENCES

Fellig, J., MacDonald, A., Meseck, E. and Laurencot, H. (1969). The metabolism of ipronidazole in the turkey. Poultry Sci., 48, 1806.

Fellig, J., Kaykaty, M., Gonzales, L., Duke, M.J. and Hecht, B.R. (1971). Clearance of ipronidazole in the turkey following administration at therapeutic levels in the drinking water. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Fellig, J., Kaykaty, M., Gonzales, L., Duke, M.J. and Hecht, B.R. (1972a). Clearance of ipronidazole in the turkey following administration at therapeutic levels in the feed. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Fellig, J., Kaykaty, M., Gonzales, L. and Eisenbeis, H.G. (1972b). Clearance of ipronidazole in the turkey following administration at 0.0625% in a premix concentrate. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Fellig, J., Kaykaty, M., Gonzales, L., Duke, M.J. and Hecht, B.R. (1972c). Clearance of ipronidazole in the turkey following administration in feed and drinking water. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Fellig, J., Kaykaty, M., Gonzales, L. and Eisenbeis, H.G. (1973a). Clearance of ipronidazole in swine following administration in feed at 100 g/ton. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Fellig, J., Kaykaty, M., Gonzales, L. and Eisenbeis, H.G. (1973b). Clearance of ipronidazole soluble powder in swine. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Fellig, J., Kaykaty, M., Gonzales, L., Eisenbeis, H.G. and Messersmith, R.E. (1975). Elimination of ipronidazole from the tissues of swine. Vet. Med./Small Animal Clinician, 70, 31-34.

Food and Drug Administration (1988). Code of Federal Regulations, 21, 477-481.

Garland, W.A. and Gonzales, L. (1975). Determination of ipronidazole (Ro 7-1554) and its metabolite (Ro 7-7916) in turkey tissue utilizing combined gas chromatography, mass spectrometry, and selective ion detection. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Garland, W.A., Hodshon, B.J., Chen, G., Weiss, G., Felicito, N.R. and MacDonald, A. (1980). Determination of ipronidazole and its principal metabolite in turkey skin and muscle by combined gas chromatography-negative chemical ionization mass spectrometry-stable isotope dilution. J. Agric. Food Chem., 28, 273-277.

Kaykaty, M. (1969). Tissue clearance of ipronidazole in turkeys. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Kaykaty, M. (1970). Tissue clearance of Ro 7-1554 and its metabolite Ro 7-7916 in swine. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Laurencot, H.J., Rose, N.K. and Kohut, J. III. (1975). In vitro metabolism of ipronidazole-2-14C by swine liver microsomes. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Laurencot, H.J., Crowley, C.A., Gonzales, L. and Campbell, J.S. (1977). The disposition and bioavailability of ipronidazole-2-14C-derived radioactivity in turkeys. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Laurencot, H., Kohut, J. III and Rose, N. (1978). The disposition of ipronidazole-derived radioactivity in swine. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

MacDonald, A. (1969). Tissue clearance of Ro 7-1554 and its metabolite Ro 7-7916 in swine. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

MacDonald, A., Castro, E., Chen, G. and Hopkins, P. (1969). Residue methodology and tissue clearance of histomonastat ipronidazole in turkeys. Poultry Sci., 48, 1837.

MacDonald, A., Chen, G., Kaykaty, M. and Fellig, J. (1971). Residue analysis of ipronidazole and its metabolite at the 2 ppb level in turkey tissue. J. Agric. Food Chem., 19, 1222-1227.

MacDonald, A. (1977a). August 17,1977, Status report: studies involving the elucidation of ipronidazole metabolism and disposition. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

MacDonald, A. (1977b). March 10, 1977, Status report: studies involving the elucidation of ipronidazole metabolism and disposition. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Messersmith, R.E., Oetjen, K.B., Hussey, F.J. and Kanning, H.H. (1973). Effect of ipronidazole on swine dysentery. Vet. Med./Small Animal Clinician, 68, 1021-1033.

Weiss, G., Rose, N. and Duke, P. (1978). Isolation and identification of a major water soluble rat fecal metabolite of ipronidazole. Unpublished report submitted to FAO by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

Weiss, G., Rose, N., Duke, P. and Williams, T.H. (1981). A major rat faecal metabolite of ipronidazole. Xenobiotica, 11, 207-215.

ISOMETAMIDIUM CHLORIDE

IDENTITY

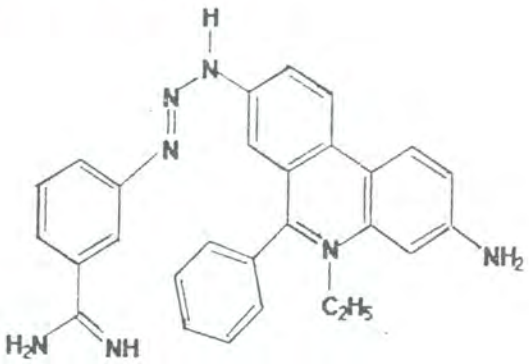
Chemical name: 3-Amino-8-[3-[3-(aminoimiinomethyl)phenyl]-1-triazenyl]-5-ethyl-6-phenylphenanthridinium chloride

8-[3-(m-amidinophenyl)-2-triazeno]-3-amino-5-ethyl-6-phenylphenanthridinium chloride

7-m-amidinophenyldiazoamino-2-amino-10-ethyl-9-phenylphenanthridinium chloride

Synonyms: Samorin and Trypamidium

Structural formula:



Molecular formula: $C_{28}H_{26}ClN_7$

Molecular weight: 496.04

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient

Appearance	Red crystals from aqueous methanol
Melting Point	Decomposes 244-245°C

Technical Active Ingredients

The commercially available products Samorin and Trypamidium have isometamidium chloride as the principle component with the remaining fraction comprising of two isomers, two analogs of a bis-species, and homidium. Isometamidium is presented as a dark reddish-brown powder with a solubility in water of 6% (w/v) at 20°C.

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Isometamidium is an antitrypansmal agent. It is used for the treatment and prevention of animal trypanosomiasis principally in cattle but also in sheep, goats, buffalo, donkeys, horses, camels and dogs. Activity has been demonstrated against Trypanosoma congolense, T.vivax, T.brucei, and T.evansi (Touratier, 1981).

Dosages

Isometamidium is prepared as a 1%, 2% or 4% (w/v) injectable aqueous suspension to be administered intramuscularly at a dose rate of 0.5 or 1.0 mg/kg body weight. Occasionally it is administered by intravenous injection (Dowler, et al., in press). For intravenous use in cattle, isometamidium is used as a 1% aqueous solution to be administered at 0.6 mg/kg.

Pharmacokinetics and Bioavailability

The distribution and elimination of isometamidium was examined in lactating dairy cattle following intramuscular injection, 1.0 mg/kg, of 14C labeled material (Bridge, et al, 1982). Peak concentrations of radio-labeled products were detected in plasma at 24 hrs (0.027 mg/ml) post dose and steadily declined to the limit of detection (0.01 mg/ml) by 29 days.

Kinabo and Bogan (1988b) investigated the absorption and distribution of isometamidium and its effect on tissues in cattle following intramuscluar injection at 0.5 mg/kg body weight. The drug was rapidly detectable in serum at a mean concentration of only 20 ng/ml and declined to concentrations of lower than 10 ng/ml within two hours. After 120 hours, serum levels of isometamidium were below the limit of detection.

The absorption of 14C-isometamidium was investigated in female rats following a single 1 mg/kg oral dose (Smith et al., 1981). Minimal absorption of the dose was observed. By day 7 after dosing, all tissues contained less than 10 ng/g, at which time 99% of the administered dose had been voided in the feces.

METHODS OF RESIDUE ANALYSIS

Earlier analytical procedures lacked sensitivity and specificity such as the spectrophotometric method described by Philips et al,, (1967) which could not detect isometamidium concentrations less that 1 mg/ml in the plasma. The HPLC method developed by Perschke and Vollner (1985) was indirect in that isometamidium was converted to homidium before assay and therefore not specific.

Most of the methods reported for isometamidium, or isometamidium isomers and analogues, are for levels in plasma or serum. Kinabo and Bogan (1988a) have developed an analytical procedure using solid-phase extraction and ion-pair reverse phase HPLC with fluorescence detection for isometamidium in bovine serum and tissues. Although the assay could detect levels of isometamidium down to 10 ng/ml in serum, the sensitivity was limited to 500 ng/g in the tissue. The authors suggest that this may be due to strong binding of isometamidium to mucopolysaccarides, nucleic acid and lipids.

METABOLISM AND RESIDUE STUDIES

Metabolism Studies

The metabolism of isometamidium has not been extensively studied. However, studies with rats (Philips, et al.,1967) and cattle (Kinabo and Bogan, 1988b) have indicated that isometamidium metabolites could not be found in the blood. The latter study indicated the injection site was the primary depot for prophylaxis. The presence of active metabolites would have been suspected if isometamidium concentrations at the injection site were as transient and low as those in serum.

Residue Studies

In the pharmacokinetic study described previously, Bridge et al. (1982) found the highest concentration of radioactivity, 73.5 mg/g, was located at the injection site 72 hours post-injection. The half-life of the injection site residues were calculated to be 39 days. The liver and kidney tissues were the other main sites of radioactivity localization. The peak concentrations of isometamidium equivalents were 7.1 and 5.8 mg/g at 72 hours with elimination half-lives of 25 and 35 days for the liver and kidney tissues

respectively. In addition to tissues, milk samples were collected and analyzed during the 90 day post injection period. Most of the samples had levels of Isometamidium which were below the limit of detection (0.01 mg/ml). However, some cows did produce positive samples (0.0138 - 0.0174 mg/ml) on single occasions from 5 to 70 days post-injection.

Isometamidium residues in calves have been reported using a sensitive HPLC method (Kinabo and Bogan, 1988b). In this study the calves were administered isometamidium at 0.5 mg/kg by intramuscular injection. Isometamidium was only detectable in the serum up to 2 hours after injection, at a mean maximum concentration of 20 ng/ml. The highest concentration of Isometamidium was detected at the injection site at 7, 21 and 42 days. The results of the various tissue assays have been tabulated in Table I.

Table I. Isometamidium residues in calves (µg/g)

Tissue	Days Post-injection 7	21	42
Injection site	1270 ± 272	315 ± 173	208 ± 94
Liver	4.80 ± 0.84	4.07 ± 0.35	.75 ± 1.41
Kidney	5.21 ± 3.36	2.98 ± 0.64	0.70 ± 0.11
Spleen	3.10 ± 1.52	2.75 ± 2.47	0.82 ± 0.07
Muscle	1.00 ± 0.02	0.87 ± 0.01	0.59 ± 0.12
Heart	0.40 ± 0.04	0.31 ± 0.08	0.20 ± 0.23

Isometamidium residues in goats treated by intramuscular and intravenous injection at a level of 0.5 mg/kg were evaluated using a spectrophotometric method (Braide and Eghianruwa, 1980). Table II shows the concentrations of isometamidium found in tissues 4 and 12 weeks after a single dose.

Table II. Isometamidium residues in goats (mg/g)

Tissue	Time (weeks)	Route of Administration: Intramuscular	Intravenous
Liver	4	5.52 ± 0.38	11.39 ± 0.61
	12	ND	6.78 ± 0.29
Kidney	4	2.51 ± 0.16	9.29 ± 0.52
	12	<1.25	3.26 ± 0.20
Spleen	4	<1.25	ND
	12	—	—
Muscle	4	ND	ND
	12	—	—
Fat	4	ND	ND
	12	—	—
Injection Site	4	2.51 ± 0.21	ND
	12	ND	—

(Note: ND = Not detected)

APPRAISAL

There are numerous deficiencies with the residue data for isometamidium; however, the resolution of these deficiencies would not decrease the residue levels experimentally determined in edible tissues. Firstly, the specifications for the commercial product indicate a minimum purity of only 70%. While colorimetric, chromatographic and

radiometric methods may detect isometamidium and the other components of the product in the tissues, the residue depletion levels varied dramatically even within a single species. Secondly, there is a lack of metabolism data to characterize what metabolites contribute significantly to the residue levels. Thirdly, the predominate conditions of use of the drug are unclear. Although it has been stated that intramuscular injection is the routine route of administration, it has been noted that intravenous injection has been used on a therapeutic basis since 1985.

The intramuscular use of isometamidium in food-producing animals results in significant and persistent residues at the injection site, liver and kidney tissues. Intravenous injection produces even higher and more persistent residues in the liver and kidney. Manufacturers of isometamidium recommend that animals not be slaughtered for human consumption within one month of treatment. Depending on the toxicological evaluation, the injection site, liver and kidneys of treated animals should be discarded prior to human consumption.

REFERENCES

Braide, V. and Eghianruwa, K.I. (1980). Isometamidium Residues in Goat Tissues after Parenteral Adminsitration. Res. Vet. Sci., 29 111-113.

Bridge, C.M., Smith, G.E., Smith, R., Templeton, R. and Yoxall, A.T. (1982). Samorin: Study in the lactating cow, of the distribution and excretion of radiolabelled drug-derived products, following the administration of a single intramuscular dose of [6-14C] Samorin. M & B Research Report R. Ph. 176.

Dowler, M.B.E., Schillinger, D. and Connor, R.J. (in press). Notes on the routine intravenous use of isometamidium in the control of bovine trypanosomiasis in the Kenya Coast. Trop Anim. Hlth Prod.

Kinabo, L.D.B. and Bogan, J.A. (1988a). Solid-phase extraction and ion-pair reversed-phase HPLC of isometamidium bovine serum and tissues. Acta Trop. (in press).

Kinabo, L.D.B. and Bogan, J.A. (1988b). Pharmacokinetic and histopathological investigation of isometamidium in cattle. Res. Vet. Sci., 44, 267-269.

Persche, M. and Vollner, L. (1985). Determination of the trypanocidal drugs Homidium, Isometamidium and Quinapyramine in bovine serum or plasma using HPLC. Acta Trop., 42, 209-216.

Phillips, F.S., Sternberg, S.S., Cronin, A.P., Sodergen, J.E. and Vidal, P.M. (1967). Physiological disposition and intracellular localisation of isometamidium. Cancer Research, 27, (1), 333-349.

Smith, J., Bridge, C.M. and Smith, G.E., (1981). Samorin: Study, in the female rat, of the distribution and excretion of radiolabelled drug-derived products after intramuscular or oral administration of a single dose of [6-14C] Samorin. M & B Research Report RES/3851.

Touratier, L. (1981). (The advantage of using isometamidium for the control at animal trypanosomasis). International Scientific Council for Trypanosomiasis Research and Control. 16th Meeting, Yaounde, Cameroon, 1979. OAU/STRC. 308-316.

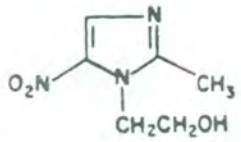
METRONIDAZOLE

IDENTITY

Chemical name: 2-methyl-5-nitroimidazole-1-ethanol
1-(2-hydroxyethyl)-2-methyl-5-nitro-imidazole

Synonyms: Flagyl and sold under at least another 20 trade names

Structural formula:



Molecular formula: $C_6H_9N_3O_3$

Molecular weight: 171.16

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: cream-colored crystals

Melting point: 158-160°C

(Windholz, 1983)

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Metronidazole is not approved for use in food-producing animals. The drug is used in human medicine for the treatment of histomoniasis and anaerobic bacterial infections.

The treatment of bovine trichomoniasis with metronidazole by the oral, intravenous or topical route has been investigated. (Gasparini, 1963)

RESIDUE DEPLETION AND METABOLISM STUDIES

General

The residue depletion and metabolism of metronidazole in food-producing animals has not been studied. However, work has been done in bacteria, rats, dogs and man.

TOTAL RESIDUE DEPLETION

Rats

Female rats weighing 200 g were administered a single 10 mg/kg dose of metronidazole labeled with 14C in the 2-position of the ring. The animals were sacrificed at 1, 4, 8, and 24 hours post dosing and various tissue samples taken. The tissues were analyzed for total radioactivity by combustion analysis. The results are presented in Table I. The

terminal $t_{1/2}$ values are approximately: muscle, 8 hours; liver, 10 hours; and kidney, 34 hours. (Ings, et. al, 1975)

Table I. Concentration of Radioactivity in Tissues of Rats At Various Times After Receiving a Single 10 mg/kg Dose of 14C-Metronidazole (ppm)

Withdrawal Time (hours)	Muscle	Liver	Kidney
1	5.71	11.04	8.57
4	2.48	6.84	5.04
8	1.12	3.41	1.98
24	0.29	1.06	1.57

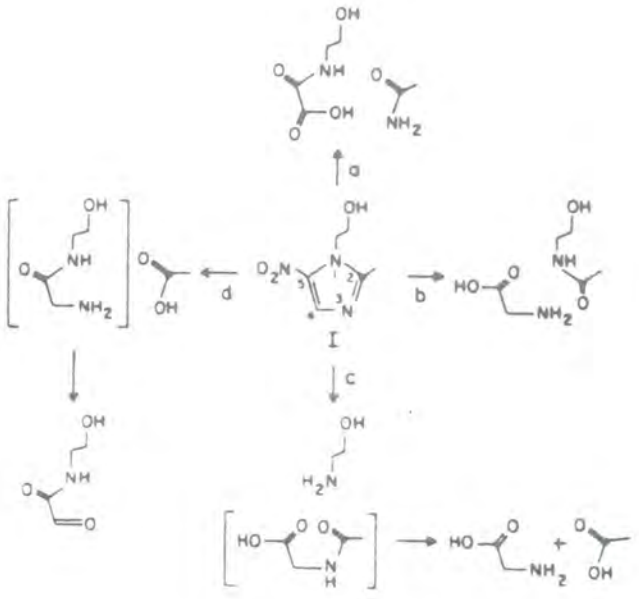
METABOLISM

Bacteria and Other in Vitro Systems

The metabolism of metronidazole by Clostridium perfringens or the microflora of the rat cecum has been examined. Metronidazole, labeled with 14C in the 1- and 2-positions of the ethanol side chain, was incubated with the rat cecal contents or C. perfringens. Under those conditions, acetamide and N-(2-hydroxyethyl)oxamic acid were identified. These metabolites are complementary in the sense that together they contain all the carbon and nitrogen atoms of metronidazole except that in the nitro group. The products could result from the cleavage of a partially reduced nitroimidazole at the 1-2 and 3-4 positions of the ring. (Koch and Goldman, 1979; Koch, et al., 1979)

Acetamide and N-(2-hydroxyethyl)oxamic acid in the above studies represented only a small fraction of the products formed from the metabolism of metronidazole. Therefore, a reductive system including milk xanthine oxidase was used in an attempt to characterize other possible products. The metabolites identified from the reduction of metronidazole mediated by xanthine oxidase included: ethanolamine, N-glycoylethanolamine, N-(2-hydroxyethyl)oxamic acid, N-acetylethanolamine, acetate, acetamide and glycine. From these results it was proposed that metronidazole could fragment in 4 ways. As shown in Figure 1, pathway a leads to N-(2-hydroxyethyl)oxamic acid and acetamide; pathway b to N-acetylethanolamine and glycine; pathway c to ethanolamine, acetate and glycine; and pathway d to N-glycoylethanolamine and acetic acid. (Crystal, et al., 1980; Goldman, et al., 1986)

Figure 1. Fragmentation Patterns of Metronidazole



Rats

The urine of rats administered a single 10 mg/kg dose of metronidazole was examined by chromatography 24 hours after treatment. Fourteen products were detected in the urine. Six of the components were identified as: metronidazole, the sulfate and glucuronide conjugates of metronidazole, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole, 1-(2-hydroxyethyl)-2-carboxyl-5-nitroimidazole, and 2-methyl-5-nitroimidazol-1-yl-acetic acid. (Ings, et al., 1975)

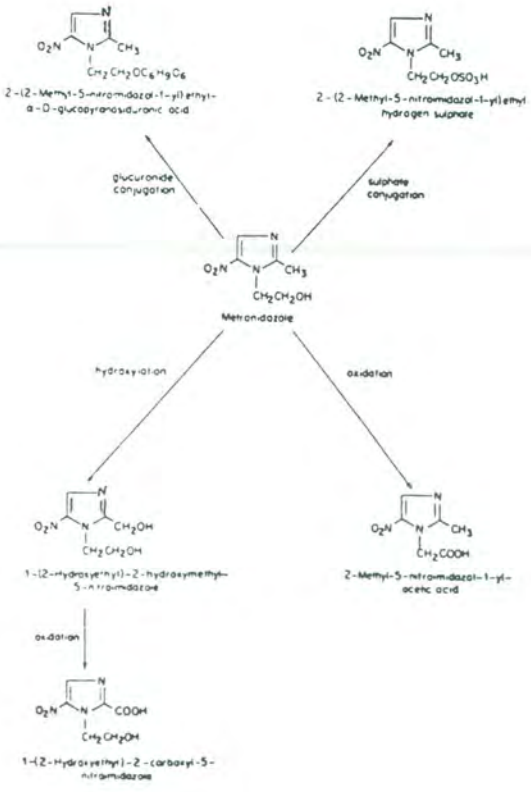
Dogs and Man

Beagle dogs were dosed by stomach tube with 100 mg/kg metronidazole, while a human was given a single oral dose of 1 g. The urine of the subjects were examined with chromatography up to nine hours post dosing. The metabolic patterns in dog and man were found to be identical. Three of the components of urine were determined to be 2-methyl-5-nitroimidazol-1-yl-acetic acid, metronidazole, and the glucuronide of metronidazole. (Ings, et al., 1966)

The urine of human subjects given 250 mg orally three times daily was collected for 24-hour periods. The urine was examined with chromatography. Six compounds were identified: metronidazole, the glucuronide of metronidazole, 2-methyl-5-nitroimidazol -1-yl-acetic acid, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole, the glucuronide of the latter dihydroxy metabolite, and 1-(2-hydroxyethyl)-2-carboxyl-5-nitroimidazole. (Stambaugh, et al., 1968)

From the above work, the metabolism of metronidazole in urine of man, dogs and rats can be summarized as shown in Figure 2.

Figure 2. Metabolism of Metronidazole in Urine of Man, Rats and Dogs



METHODS OF RESIDUE ANALYSIS

Because there are no approvals for metronidazole in food-producing animals, no specific methods of analysis for residues of metronidazole in edible tissues have been reported. Nevertheless, it is anticipated that polarographic assays, sensitive to the low ppb range, could be adapted for metronidazole. For example, some preliminary work has been done with metronidazole using a differential pulse polarographic assay with a claimed limit of detection of 1 ppb. (Ohst, 1987)

APPRAISAL

Metronidazole is not approved for use in food-producing animals and, consequently, specific methods of analysis are unavailable. Although relevant studies have not been done, the routes available for the metabolism of metronidazole in food-animals may be anticipated from the work done in rats, dogs and man.

REFERENCES

Chrystal, E.J.T., Koch, R.L. and Goldman, P. (1980). Metabolites from the reduction of metronidazole by xanthine oxidase. Mol. Pharmacol., 18, 105-111.

Gasparini, G., Vaghi, M. and Tardani, A. (1963). Treatment of bovine trichomoniasis with metronidazole (8823 R.P.). Vet. Rec., 75, 940-943.

Goldman, P., Koch, R.L., Yeung, T-C., Chrystal, E.J.T., Beaulieu, B.B. Jr., McLafferty, M.A. and Sudlow, G. (1986). Comparing the reduction of nitroimidazoles in bacteria and mammalian tissues and relating it to biological activity. Biochem. Pharmacol., 35, 43-51.

Ings, R.M.J., Law, G.L. and Parnell, E.W. (1966). The metabolism of metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole). Biochem. Pharmacol., 15, 515-519.

Ings, R.M.J., McFadzean, J.A. and Ormerod, W.E. (1975). The fate of metronidazole and its implications in chemotherapy. Xenobiotica, 5, 223-235.

Koch, R.L., Chrystal, E.J.T., Beaulieu, B.B. Jr. and Goldman, P. (1979). Acetamide-a metabolite of metronidazole formed by the intestinal flora. Biochem. Pharmacol., 28, 3611-3615.

Koch, R.L. and Goldman, P. (1979). The anaerobic metabolism of metronidazole forms N-(2-hydroxyethyl)oxamic acid. J. Pharmacol. Exp. Ther., 208, 406-410.

Ohst, E. (1987). Development of a method for detecting nitroimidazole residues in pig muscle. Report No. 1186. Submitted to FAO by BASF Aktiengesellschaft, Ludwigshafen.

Stambaugh, J.E., Feo, L.G. and Manthei, R.W. (1968). The isolation and identification of the urinary oxidative metabolites of metronidazole in man. J. Pharmacol. Exp. Ther., 161, 373-381.

Windholz, M. ed. (1983). The Merck Index 10th Edition. Rahway, N.J., Merck and Co.

RONIDAZOLE

IDENTITY

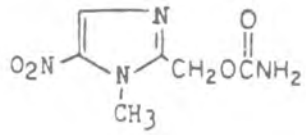
Chemical name: 1-methyl-5-nitroimidazole-2-methanol carbamate (ester)

carbamic acid (1-methyl-5-nitro-imidazol-2-yl)methyl ester

1-methyl-2-[(carbamoyloxy)methyl]-5-nitroimidazole
(1-methyl-5-nitroimidazole-2-yl)-methyl carbamate

Synonyms: Ridzol
Dugro
MCMN

Structural formula:



Molecular formula: $C_6H_8N_4O_4$

Molecular weight: 200.16

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: pale yellow crystals

Melting point: 167-169°C

(Windholz, 1983)

RESIDUES IN ANIMALS AND THEIR EVALUATION

CONDITIONS OF USE

General

Ronidazole is used in turkeys for the prevention and treatment of histomoniasis. The drug is also used in swine for the prevention and treatment of swine dysentery.

Dosages

Ronidazole is administered to swine at 0.006-0.008% in the feed for prevention and at 0.012% in the feed or 0.006% in the drinking water for 3 to 5 days for treatment.

The drug is administered to turkeys at 0.006-0.009% in the feed for prevention and at 0.012% in the feed or 0.004-0.006% in the drinking water for 7-14 days for treatment.

RADIOLABELED RESIDUE DEPLETION STUDIES

Turkeys

Three-week-old turkey poults were fed diets containing 0.006% 14C-ronidazole for four days. The radioactive forms of the drug used in the experiment had label in the N-methyl group or in the 2-position of the ring. The concentration of total residue of ronidazole was measured at various withdrawal times. The results are given in Table I. Beyond 21 days the levels of radioactivity in tissues of treated animals were indistinguishable from those in tissues of control animals. The residue depletion of ronidazole was not significantly different when birds were dosed with one radioactive form of ronidazole or the other. (Rosenblum, et al., 1972)

Table I. Concentration of Total Residue in Tissues of Turkeys Treated With 0.006% Ronidazole in the Diet (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	3.0	4.5	4.7	-
2	0.28	0.5	0.73	0.37
5	0.09	0.18	0.4	-
10	0.26	0.05	0.14	-
14	0.03	0	0.07	-
21	0.04	0	0	-

In a second experiment turkeys were maintained on a diet containing 0.006% 14C-ronidazole (labeled in the N-methyl or the 2-position of the ring) and sacrificed at various withdrawal times. Tissues from the birds were analyzed for total residue, as well as parent drug and its metabolite, 2-hydroxymethyl-1-methyl-5-nitroimidazole, HMMNI). Ronidazole and the metabolite were determined with tlc and electrophoretic methods. The results are shown in Table II. The results of this study are similar to those described above both in terms of (1) the total residue values determined at the various withdrawal times, and (2) the comparability of the data obtained with each labeled form of ronidazole. (Rosenblum, et al., 1972) (Rosenblum, 1977)

Table II. Concentration of Total Residue, Ronidazole, and HMMNI in Turkeys Treated at 0.006% in the Feed (ppm)

Treatment	Tissue	Total Residue	Ronidazole	HMMNI
3 days on drug	Kidney	4.00	<0.03	0.0
N-methyl-14C	Liver	4.15	<0.02	0.0
0-withdrawal	Muscle	2.58	1.5	0.1
3 days on drug	Kidney	4.43	0.09	0.0
ring-2-14C	Liver	3.77	0.01	0.0
0-withdrawal	Muscle	2.05	1.6	0.03
3 days on drug	Kidney	0.9	0.0	0.0
ring-2-14C	Liver	0.38	0.0	0.0
2-day withdrawal	Muscle	0.15	0.007	<0.01
3 days on drug	Kidney	0.44	0.0	0.0
ring-2-14C	Liver	0.13	0.0	0.0
5-day withdrawal	Muscle	0.07	0.0	0.0

Swine

Swine weighing 20-30 kg and mixed as to sex were dosed with 14C-ronidazole labeled in the N-methyl group once daily for three days in a feed slurry. The dose was 7 mg/kg, which would be equivalent to a dose obtained from feed containing 0.006% ronidazole. Three animals were sacrificed at 0 (6 hours), 3, 7, 14, 28 and 42 days of withdrawal. Tissue samples taken from the animals were assayed for total radioactivity by combustion analysis. The results of the assays are given in Table III. (Wolf, et al., 1983a)

Table III. Total Residue in Tissues of Swine Treated With 14C-Ronidazole at 7 mg/kg (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	6.32	10.63	9.37	1.46
3	0.49	1.53	1.22	0.30
7	0.52	1.15	0.85	0.25
14	0.35	0.44	0.27	0.15
28	0.18	0.10	0.09	0.06
42	0.13	0.06	0.05	0.05

In another experiment, four barrows weighing approximately 20 kg were dosed with 14C-methyl-labeled ronidazole at 6.7 to 12 mg/kg for three days. The animals were sacrificed at 6 or 72 hours after the last dose and tissue samples were taken for radioassay. The results of the assays are presented in Table IV. (Wolf, et al., 1983a)

Table IV. Total Residue in Tissues of Swine Treated With 14C-Methyl-labeled Ronidazole (ppm)

Animal No. (withdrawal)	Dose mg/kg	Muscle	Liver	Kidney	Fat
19 (6 hours)	6.7	5.0	7.8	7.9	2.5
21 (6 hours)	12	8.6	12.3	11.9	1.3
17 (72 hours)	9.2	0.5	1.6	1.1	0.4
22 (72 hours)	12	1.1	2.4	2.5	0.2

Bioavailability of Swine Muscle Residue in Rat

Muscle tissue, obtained from pigs dosed with 14C-methyl-labeled ronidazole daily for 3 days and sacrificed 7 days after the last feeding, was homogenized with four volumes of water and freeze dried. The freeze-dried muscle was mixed with Purina rat chow at a ratio of 4 parts of muscle tissue (containing radioactivity equivalent to 16 μg of ronidazole) and 5 parts of chow. A control diet was prepared by blending 16 μg of $^{14}CH_3$-ronidazole with freeze-dried muscle from unmedicated pigs. Rats were fed with 18 g of the chow-muscle mixture late in the day for two days. Radioactivity was measured in the whole carcass minus skin and the GI tract.

The overall recovery of radioactive ronidazole was 102.78% for animals dosed with muscle from ronidazole-treated swine and 91.33% for those dosed with muscle spiked with ronidazole. The breakdown for the recovery of the radioactivity is given in Table V. (Wolf, et al., 1984a)

Table V. Recovery of Radioactivity from Rats Dosed with Labeled Ronidazole or Muscle From Swine Treated With Labeled Ronidazole, Expressed as a Percent of Dose

	Substance Dosed	
Sample	Ronidazole	Swine Muscle Residue
urine	44.69	26.39
feces	39.12	25.29
expired gas	3.31	11.20
GI tract	1.97	18.00
carcass	2.24	21.90
Total	91.33	102.78

It is of interest that the carcass and expired air of the muscle-treated rats contain a higher percentage of the radioactivity than do those of rats treated with ronidazole. Furthermore, it was found that 92% of the methylamine-releasing residues (i.e., compounds such as ronidazole or derivatives containing the N-methyl group) in the swine muscle was recovered in rat urine and feces, with <0.5% in the carcass. The sponsor of the drug believes that the results indicate retained radioactivity in tissues reflects more the

incorporation of the label into endogenous substances than the formation of 'drug-related' bound residue. (Wolf, et al., 1984a)

RESIDUE DEPLETION STUDIES

Swine

Two groups of three pigs, mixed as to sex and weighing about 120 lb, were administered 0.012% ronidazole through the drinking water for 7 days. One group was sacrificed with a 1-day withdrawal, the other with a 3-day withdrawal. Samples of muscle, liver, kidney and fat from each treated animal were taken for analysis. A differential pulse polarographic method with a sensitivity of 2 ppb was used to assay for ronidazole. Muscle tissues from animals sacrificed with a 1-day withdrawal were the only samples to show detectable residues of ronidazole, averaging 24 ppb. (Downing, et al., 1973)

Six groups of three pigs, mixed as to sex and weighing about 75 lb, were administered 0.012% ronidazole through the drinking water for 7 days. Groups were sacrificed at 0, 1, 3, 5, 7, or 9 days post dosing. Samples of muscle, liver, kidney and fat from each treated animal were taken for analysis with the differential pulse polarographic method described above. Detectable amounts of ronidazole were obtained on days 0 and 1 of withdrawal only; at all other withdrawal periods no ronidazole was found. The results for 0 and 1 day of withdrawal are summarized in Table VI. (Downing, et al., 1973) (Cala, et al., 1976)

Table VI. Concentrations of Ronidazole in Tissues of Swine Treated at 0.012% Through the Drinking Water (ppb) (ND = no detectable residue)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	3010	ND	14	58
1	80	ND	ND	ND

Six groups of three pigs, mixed as to sex and weighing about 25 lb, were fed a diet containing 0.009% ronidazole for 7 weeks (up until the pigs weighed about 75 lb). Groups were sacrificed at 0, 1, 3, 5, 7 or 9 days of withdrawal. Samples of tissues were assayed for ronidazole with the differential pulse polarographic method. As above, detectable amounts of ronidazole were obtained on days 0 and 1 of withdrawal only. The results are presented in Table VII. (Downing, et al., 1974)

Table VII. Concentrations of Ronidazole in Tissues of Swine Treated at 0.009% in the Feed For 7 Weeks (ppb) (ND = no detectable residue)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	612	ND	16	20
1	152	ND	6	4

Another experiment was conducted in a manner identical to that above, except the swine were fed the medicated diet for 12 weeks (up until the pigs weighed 175 lb). The results for this study are given in Table VIII. (Downing, et al., 1974)

Table VIII. Concentration of Ronidazole in Tissues of Swine Treated at 0.009% in the Feed for 12 Weeks (ppb) (ND = no detectable residue)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
0	409	ND	1.3	4
1	9	ND	ND	ND

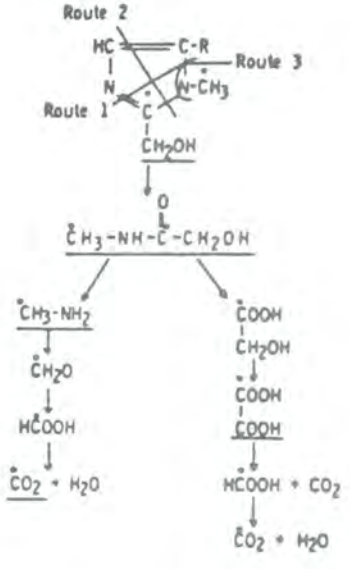
METABOLISM STUDIES

Turkeys

In a study of the metabolism of ronidazole in turkeys, birds were administered 0.006% 14C-ronidazole (labeled in the C-2 position of the ring or in the N-methyl group) through the feed for 3 days. Samples of tissues were analyzed for metabolites using paper electrophoresis and thin layer chromatography. Ronidazole and its metabolite HMMNI were identified in muscle from 0-withdrawal birds only. Glucuronide conjugates of ronidazole and HMMNI in muscle and urine were not detected. Analysis of the aqueous soluble extracts of liver, which contain approximately 80% of the total liver radioactivity, showed the presence of 14C-N-methylglycolamide, 14C-oxalic acid (from birds dosed with 2-14C-ronidazole), and 14C-methylamine. Evidence was also accumulated for the presence of various acids, including fumaric, succinic, glycolic, malic, α-ketoglutaric, and citric, in the extracts of liver from treated birds. Further, radioactivity was seen to be associated with amino acids in pooled liver samples from treated turkeys. The authors of this work contend that the bulk of radioactivity in the liver, therefore, proved to represent a wide variety of simple known substances commonly present in normal tissue (and, consequently, not of toxicological significance). (Rosenblum, et al., 1972) (Rosenblum, 1977)

The proposed pathways for the metabolism of ronidazole in turkeys are shown in Figure 1. Hydrolysis of the carbamate group leaves HMMNI. Ring scission may occur by several routes. Routes 1 and 2 lead to N-methylglycolamide, which in turn can be further metabolized to oxalic acid, methylamine and carbon dioxide. The nitro group is represented as R, because its exact status remains unknown. However, it has been suggested that the nitro group may simply hydrolyze or be reduced to the amine, which in turn can hydrolyze to the hydroxy group. (Rosenblum, et al., 1972)

Figure 1. Proposed Routes of Metabolism of Ronidazole in Turkeys.



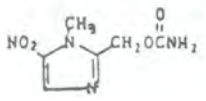
Swine

Studies similar to those carried out in turkeys were conducted in swine. Two 10-week-old barrows weighing approximately 20 kg were fed 14C-ronidazole, labeled in the N-methyl group, mixed in a slurry of feed and water at a rate of 6.7 mg/kg or 9.2 mg/kg per day for 3 days. One pig was sacrificed at 4 hours post last dose and the other at 72 hours following the final dose. Approximately 70-80% of the total radioactivity was recovered in urine, feces, GI tract contents and tissues. The authors speculate that the remainder of the radioactivity could be lost via exhalable material (methylamine, for example).

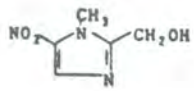
The amount of residue that was unextractable from muscle and liver of treated animals increased with time. Thus for the animal sacrificed at 4 hours, 74% of the radioactivity in liver and 16% in muscle was water soluble, while 28% and 14%, respectively, were insoluble. For the pig sacrificed at 72 hours, 26% of the radioactivity in liver and 27% in muscle was water soluble, while 71% and 65%, respectively, were insoluble. Moreover, when the cell components in tissue samples of the 72-hour sacrificed pig were examined, it was found that 53.6% of the total radioactivity in liver was associated with protein and about 10% each being distributed with the nucleic acid and lipid fractions. In muscle of that same animal, 58.3% of the total radioactivity was associated with protein and about 6% each in the nucleic acid and lipid fractions. The increase in the proportion of the radioactivity in the insoluble residue was taken as reflecting the incorporation of radioactive substances into macromolecular cellular constituents which have a relatively longer biological half-life than the lower molecular weight compounds present in the water-soluble fraction.

The examination of urine, muscle and liver for metabolites of ronidazole determined the presence of four components: ronidazole, HMMNI, imidazole and 1-methyl-2-carbamoyloxymethyl 1-5-acetamido-imidazole. With the exception of ronidazole at 4500 ppb and HMMNI at 95 ppb in muscle from the 4-hr sacrificed pig, none of the above compounds was present above 5 ppb in muscle or liver of either treated animal. In urine, the only two nitro-containing compounds found were ronidazole and HMMNI. The structures of the above compounds are shown in Figure 2. (Smith, et al., 1975)

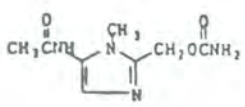
Figure 2. Metabolites of Ronidazole Found in Tissues of Treated Swine.



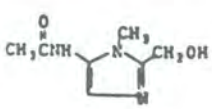
Ronidazole



1-methyl-2-hydroxymethyl-5-nitroimidazole



1-methyl-2-carbamoyloxymethyl-5-acetamidoimidazole



1-methyl-2-hydroxymethyl-5-acetamidoimidazole

The total residue depletion data in Table III show that, after a period of rapid elimination (day 0 to day 3 of withdrawal), residues persist in tissues for up to 42 days. These persistent residues were thought to be associated with cellular macromolecules. In fact, it was determined that about 60% of the radioactivity in muscle at 7 days of withdrawal was present in the protein fraction. Furthermore, that percentage did not change appreciably at 42 days of withdrawal. In order to estimate the amount of the persistent residue that was not due to incorporation of labeled material into endogenous substances, the researchers hydrolyzed tissue samples under acidic conditions to generate methylamine. Under those experimental conditions standards such as 14C-N-methyl-labeled ronidazole and dimetridazole gave quantitative yields of methylamine. Therefore, all metabolic products which have not been degraded to a single carbon fragment would yield radioactive methylamine. Tissue samples taken from animals sacrificed at 0 withdrawal showed that approximately 90% of the radioactivity present in muscle and 70% of that in liver liberates radioactive methylamine. Three days later, less than 30% of muscle radioactivity liberated methylamine. At this time, most of the methylamine liberating residues are present in the protein fraction. Since only 8-10% of the total radioactivity present in the 0-withdrawal sample remains after 3 days, and there is little change at 7

days after the last dose, it is clear that most of the methylamine generating substances have been eliminated within 3 days after the last dose. The authors concluded that the 70-80% of the residue which does not liberate methylamine represents incorporation of the 14C into endogenous substances. (Wolf, et al., 1983a)

Rats

Rats weighing 180-200 g were administered a single 10 mg/kg dose of ronidazole, labeled in one of four positions with 14C. Ronidazole was prepared with label in the N-methyl group; in the methylene group at the 2-position; in the 4- and 5-positions of the ring; and in the carbonyl group. Groups of three rats were sacrificed and tissues analyzed at 2, 4, 7, and 11 or 14 days after dosing. The results of the analyses are presented in Table IX.

Table IX. Concentration of Total Residue in Tissues of Rats Dosed with 14C-Ronidazole at 10 mg/kg (ppm)

Label Site	Withdrawal Time (days)	Liver	Muscle	Kidney	Fat
$-CH_3$	2	0.33	0.31	0.48	0.14
	4	0.27	0.26	0.41	0.13
	7	0.16	0.23	0.26	0.09
	11	0.11	0.18	0.17	0.07
$-CH_2-$	2	0.22	0.18	0.35	0.23
	4	0.10	0.13	0.26	0.14
	7	0.07	0.10	0.11	0.08
	14	0.02	0.05	0.04	0.06
4,5-ring	2	0.18	0.17	0.26	0.04
	4	0.10	0.11	0.14	0.03
	7	0.06	0.07	0.07	0.02
	14	0.03	0.06	0.04	0.02
-CO	2	0.40	0.12	0.19	0.07
	4	0.25	0.11	0.17	0.11
	7	0.12	0.07	0.08	0.07
	14	0,04	0.06	0.04	0.05

The varying levels of radioactivity in tissues from animals treated with different labels was taken by the authors as a clear indication that all of the residue cannot contain an intact N-methylimidazole nucleus. If the tissue residues contained only substances with an intact imidazole nucleus each label site should produce the same level of residues in each tissue at each analysis time, and parameters such as the $t_{1/2}$ of the residue would be identical.

The use of the methylamine generation test to determine the extent that the total residue may contain an intact imidazole nucleus, gave estimates of 10-30% of the total residue in muscle and liver at 7 and 11 days post dosing. These results are similar to those obtained for swine. (Wolf, et al., 1984b)

It is noteworthy that acetamide was identified as a metabolite in the urine of rats treated with ronidazole at 10 mg/kg. Acetamide, a known carcinogen, was found to be a breakdown product of metronidazole and its formation from ronidazole and dimetridazole seemed quite possible. (Alvaro, 1982)

EVALUATION OF THE PERSISTENT RESIDUE

General

The residue chemistry data summarized above gave evidence of persistent residues in the tissues of rats, turkeys and swine. Despite the good effort made in the metabolism studies that showed that ronidazole was extensively metabolized *in vivo*,

the exact nature of the total residue was undetermined. The data did indicate, however, that approximately 50-60% of the radioactivity remaining in tissues existed as protein-bound residues. Although a significant portion of that radioactivity was considered to be due to endogenous substances (and, therefore not of toxicological concern), the possibility that some of the radioactivity represented protein-bound metabolites containing the intact imidazole ring could not be discounted. Therefore, the sponsor of ronidazole, Merck Sharp and Dohme, undertook a series of studies aimed at determining the nature of the bound residue, the mechanism of its formation and the toxicological potential of ronidazole-bound residues.

The overall strategy for evaluating the safety of ronidazole protein-bound residues involved three phases. First, rat liver microsomes in an in vitro covalent binding system were used to study the mechanism of bound residue formation. Second, in vivo studies were conducted in the rat to permit a comparison with the residues formed in vitro and in swine to allow a comparison with the residue formed in the rat. Third, the Ames test was used to assess the potential mutagenicity of the bound residue. The results of those studies are highlighted below. (West, et al., 1982a) (West, et al., 1982b) (Miwa, et al., 1982) (Wolf, et al., 1983b) (Wislocki, et al., 1984a) (Wislocki, et al., 1984b) (Miwa, et al., 1984)

In Vitro Rat Studies

A number of important observations were made in the in vitro work:

(1) Anaerobic conditions are required for maximum binding and high oxygen concentrations inhibit covalent binding. Protein was found to be the primary binding target, with nucleic acid a very poor competitor. It was further observed that for every 20 molecules of ronidazole metabolized, only one (5%) alkylates microsomal protein. (West, et al., 1982a) (West, et al., 1982b)

(2) Cytochrome P-450 and P-450 reductase, in the presence of NADPH, catalyzes the reductive activation of ronidazole. (West, et al., 1982b)

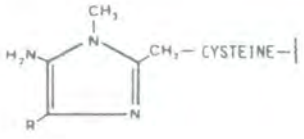
(3) The primary target of protein alkylation (nonspecific) is the cysteine thiol group. (West, et al., 1982b) (Miwa, et al., 1982)

(4) The major protein adduct retained the imidazole nucleus, but lost the carbamate group and the proton at the C-4 position. (Miwa, et al., 1982) (Miwa, et al., 1984)

(5) Although attachment of the cysteine thiol group can occur at the 2-methylene group or the 4-position of the ring, the studies suggest that the adduct occurs primarily at the 2-methylene group (see Figure 3 below). (Wislocki, et al., 1984a)

(6) The results of the studies suggest that the hydroxylamine derivative of ronidazole is the reactive species for covalent binding. (Miwa, et al., 1986b)

Figure 3. Generalized Structure for the Protein-bound Ronidazole Adduct.



In Vivo Rat and Swine Studies

Ronidazole labeled in different positions was administered to rats and swine. The animals were sacrificed at 6 hours post dosing and the protein-bound residues from liver and muscle were isolated for methylamine release experiments, oxalic acid formation determinations and chromatographic properties examination. Comparisons of the protein-bound residue from the in vitro rat microsomal system and the in vivo rat and swine systems, established the following: (1) HPLC analysis of acid hydrolyzed in

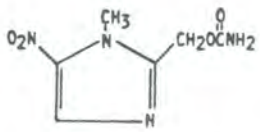
vitro and in vivo protein-bound residues obtained from 14C-ronidazole labeled in the 2-methylene position gave nearly identical radiochromatographic profiles. (2) Acid hydrolysis of the in vitro and in vivo residue samples from animals dosed with 14C-ronidazole labeled in the N-methyl group generated similar amounts of methylamine (rat microsome, 97%; rat in vivo, 76%; swine liver, 94% and muscle, 86%). Similarly, acid hydrolysis of samples from animals dosed with 4,5-ring-labeled ronidazole gave similar amounts of oxalic acid (rat microsomes, 10%; rat in vivo, 8.7%; swine liver, 9% and muscle, 6.5%). (3) All three protein-bound residue samples retain the intact imidazole nucleus but have lost the proton at the C-4 position. (Miwa, et al., 1984)

Toxicological Evaluation of Ronidazole-Protein Adduct

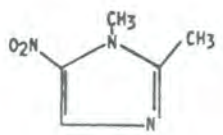
Because ronidazole is mutagenic in the Ames test, the systematic study of the mutagenicity of ronidazole and its derivatives was considered to be a logical approach to the assessment of the toxicological potential of the protein-bound residues. In particular, the Ames test was used to assess the activity of compounds structurally related to the bound residue to establish a structure-activity relationship, of free and bound microsomal metabolites of ronidazole, and of digests of protein-bound ronidazole adducts.

The mutagenic activity of a number of ronidazole-related compounds in the Ames test was examined. As shown in Figure 4, the removal of the carbamoyl group of ronidazole reduces the mutagenic activity by a factor of 10; substitution of an alkyl group at the 4-position further decreases the activity by another factor of 10. The reduction of the nitro group totally eliminates mutagenic activity. Based on these results and the observation that monocysteine-ronidazole adduct shows no mutagenic activity, it was concluded that the protein adduct would not be mutagenic. (Wislocki, et al., 1984b)

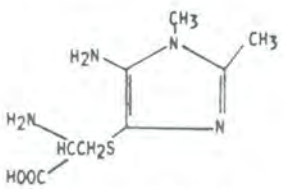
Figure 4. Mutagenic Activity of Ronidazole-related Compounds in the Ames Test Relative to Ronidazole (Taken as 100%)



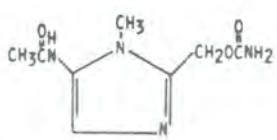
RONIDAZOLE (100%)



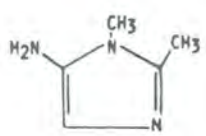
DIMETRIDAZOLE (10%)



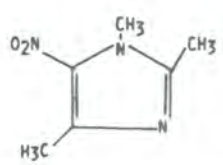
MONOCYSTEINE-RONIDAZOLE ADDUCT (0%)



N-ACETYLAMINO-1-METHYLIMIDAZOLE 2-METHANOL CARBAMATE (0%)



5-AMINO-1,2-DIMETHYLIMIDAZOLE (0%)



5-NO_2-1,2,4-TRIMETHYLIMIDAZOLE (1%)

Reduced metabolites from incubations of ronidazole were tested directly for mutagenicity in the Ames test without isolation to ensure that all metabolites were examined. Thus, ronidazole was incubated anaerobically with rat liver microsomes, NADPH-generating system, and cysteine for a prolonged period to achieve >99% metabolism. The supernatant fraction of the incubation, which contained residual ronidazole, reduced metabolites and their breakdown products, was separated from the microsomes and tested in the Ames test with and without the S-9 fraction. The supernatant was found to have little mutagenic activity and that was attributable to the residual ronidazole. These results were taken as demonstrating that the reduced metabolites and the cysteine adducts derived from ronidazole are non-mutagenic and that they cannot be activated by liver enzymes to mutagenic species. (Wislocki, et al., 1984b)

An attempt was made to determine whether mutagenic products could be released from the in vitro ronidazole-protein residues. Protease-treated ronidazole-bound residues were examined in the Ames test that had been modified to increase its sensitivity. No mutagenic activity was detected. In contrast, the addition of a few μg of ronidazole to the assay system containing maximum amounts of hydrolyzed protein samples produced mutagenic activity. (Miwa, et al., 1986a)

METHODS OF RESIDUE ANALYSIS

A combination tlc and differential pulse polarography method for ronidazole in the edible tissues of swine has been described. The assay involves extraction, purification with tlc and polarographic analysis. The method has a level of sensitivity of 2 ppb. HMMNI, the major metabolite of ronidazole, is poorly extracted in the initial step and then separated from ronidazole with tlc. The method, therefore, is specific for ronidazole. (Cala, et al., 1976)

APPRAISAL

The use of ronidazole at permitted concentrations in the feed of turkeys for 3 days produces residues that deplete below detectable levels by 5 days of withdrawal. The level of sensitivity of the assay used for ronidazole and HMMNI, though not specified, appeared to be in the 20-40 ppb range.

Tissues of swine treated with ronidazole through the feed or drinking water at prophylactic or therapeutic levels were free of detectable levels at 2 days of withdrawal. The level of sensitivity of the assay used for ronidazole was 2 ppb.

Although the residue depletion studies in turkeys and swine demonstrate that residues of parent ronidazole fall below detectable levels by 5 and 2 days of withdrawal, respectively, the radiotracer work shows that total residues persist to at least 21 days in turkeys and 42 days in swine. The residue chemistry data are not adequate for establishing a relationship between the total residue and a compound measured with a chemical assay.

The metabolism work indicates the two major pathways characteristic of the 5-nitroimidazoles: the oxidative and reductive routes. The reductive pathway, known to lead to ring scission and fragmentation, has also been implicated as producing the reactive intermediate that forms protein-bound residues containing the intact imidazole ring.

The most intriguing aspect of the data submitted for ronidazole unquestionably is the bound residue issue. Merck has conducted extensive research aimed at sensibly dealing with the toxicological significance of bound residues. In particular, the experimentation attempts to permit the prediction that the protein-bound residue would not be mutagenic and, therefore, not of significant toxicological concern.

The above work on the bound residue needs to be carefully considered in relation to the tolerance for ronidazole in edible tissues of food-producing animals to decide whether the bound residue can be reasonably assessed, and, consequently, discounted from the total residue of toxicological concern. The discounting of bound residues is particularly important for ronidazole, and the 5-nitroimidazoles generally, because it appears impossible to develop suitable regulatory analytical procedures to monitor for residues beyond a few days post treatment.

REFERENCES

Alvaro, R.F. (1982). May 4, 1982, Memorandum: Identification of one of the metabolites of ronidazole in rat urine as acetamide. Unpublished report submitted to FAO by MSD Research Laboratories, Lauterbach, Federal Republic of Germany.

Cala, P.C., Downing, G.V. Jr., Michielli, R.F. and Wittick, J.J. (1976). Determination of ronidazole in swine tissues by differential pulse polarography. J. Agric. Food Chem., 24, 764-767.

Downing, G.V. Jr., Ott, W.H., Cala, P.C., Michielli, R.F., Olson, G., Van Iderstine, A.A. and Cobb, W.R. (1973). Ronidazole: method for analysis of ronidazole and tissue residue studies in swine. Unpublished report submitted to FAO by MSD Research Laboratories, Lauterbach, Federal Republic of Germany.

Downing, G.V. Jr., Olson, G., Cala, P.C., Cobb, W.R., Michielli, R.F., Otto, K.G. and Van Iderstine, A.A. (1974). Ronidazole: tissue residue study in swine [0.009% ronidazole in the feed during growing and finishing periods]. Unpublished report submitted to FAO by MSD Research Laboratories, Lauterbach, Federal Republic of Germany.

Miwa, G.T., West, S.B., Walsh, J.S., Wolf, F.J. and Lu, A.Y.H. (1982). Drug residue formation from ronidazole, a 5-nitroimidazole. III. Studies on the mechanism of protein alkylation in vitro. Chem-Biol. Interact., 41, 297-312.

Miwa, G.T., Alvaro, R.F., Walsh, J.S., Wang, R. and Lu, A.Y.H. (1984). Drug residue formation from ronidazole, a 5-nitroimidazole. VII. Comparison of protein-bound products formed in vitro and in vivo. Chem-Biol. Interact., 50, 189-202.

Miwa, G.T., Wislocki, P., Bagan, E., Wang, R., Walsh, J.S. and Lu, A.Y.H. (1986a). Studies on the mechanism of activation and mutagenicity of ronidazole, a 5-nitroimidazole. In: Biological Reactive Intermediates. III. Plenum Publishing Corporation, pp. 527-535.

Miwa, G.T., Wang, R., Alvaro, R., Walsh, J.S. and Lu, A.Y.H. (1986b). The metabolic activation of ronidazole [(1-methyl-5-nitroimidazole-2-yl)-methyl carbamate] to reactive metabolites by mammalian, cecal bacterial and T. foetus enzymes. Biochem. Pharmacol., 35, 33-36.

Rosenblum, C., Trenner, N.R., Buhs, R.P., Hiremath, C.B., Koniuszy, F.R. and Wolf, D.E. (1972). Metabolism of ronidazole (1-methyl-5-nitroimidazol-2-ylmethyl carbamate). J. Agric. Food Chem., 20, 360-371.

Rosenblum, C. (1977). Non-drug-related residues in tracer studies. J. Toxicol. and Environ. Health, 2, 803-814.

Smith, G.E., Wolf, F.J. and Baer, J.E. (1975). Ronidazole: metabolism in swine. Unpublished report submitted to FAO by MSD Research Laboratories, Lauterbach, Federal Republic of Germany.

West, S.B., Wislocki, P.G., Fiorentini, K.M., Alvaro, R., Wolf, F.J. and Lu, A.Y.H. (1982a). Drug residue formation from ronidazole, a 5-nitroimidazole. I. Characterization of in vitro protein alkylation.

West, S.B., Wislocki, P.G., Wolf, F.J. and Lu, A.Y.H. (1982b). Drug residue formation from ronidazole, a 5-nitroimidazole. II. Involvement of microsomal NADPH-cytochrome P-450 reductase in protein alkylation in vitro. Chem-Biol. Interact., 41, 281-296.

Windholz, M. ed. (1983). The Merck Index 10th Edition. Rahway, N.J., Merck and Co.

Wislocki, P.G., Bagan, E.S., VandenHeuvel, W.J.A., Walker, R.W., Alvaro, R.F., Arison, B.H., Lu, A.Y.H. and Wolf, F.J. (1984a). Drug residue formation from ronidazole, a 5-nitroimidazole. V. Cysteine adducts formed upon reduction of ronidazole by dithionite or rat liver enzymes in the presence of cysteine. Chem-Biol. Interact., 49, 13-25.

Wislocki, P.G., Bagan, E.S., Cook, M.M., Bradley, M.O., Wolf, F.J. and Lu, A.Y.H. (1984b). Drug residue formation from ronidazole, a 5-nitroimidazole. VI. Lack of mutagenic activity of reduced metabolites and derivatives of ronidazole. Chem-Biol. Interact., 49, 27-38.

Wolf, F.J., Baylis, F.P., Smith, G.E., Rosenblum, C., Meriwether, H.T., Alvaro, R.F., Wolf, D.E., Koniuszy, F.R. and Jacob, T.A. (1983a). Disposition of ronidazole by swine. 1. Radiocarbon content of tissues. J. Agric. Food Chem., 31, 559-564.

Wolf, F.J., Alvaro, R.F., Fiorentini, K., Green, M., Lu, A.Y.H., Miwa, G.T., Wislocki, P.G., Koch, R.T., McLafferty, M.A. and Goldman, P. (1983b). Drug residue formation from ronidazole, a 5-nitroimidazole. IV. The role of the microflora. Chem-Biol. Interact., 45, 7-14.

Wolf, F.J., Alvaro, R., Steffens, J.J., Wolf, D.E., Koniuszy, F.R., Green, M.L. and Jacob, T.A. (1984a). Tissue residues due to ronidazole: bioavailability of residues in swine muscle on ingestion by the rat. J. Agric. Food Chem., 32, 711-714.

Wolf, F.J., Alvaro, R., Chapin, L.R., Green, M.L., Wolf, D.E., Koniuszy, F.R., Steffens, J.J., and Jacob, T.A. (1984b). Tissue residue studies with ronidazole: effect of label site on total radioactivity content of rat tissues. J. Agric. Food Chem., 32, 706-711.

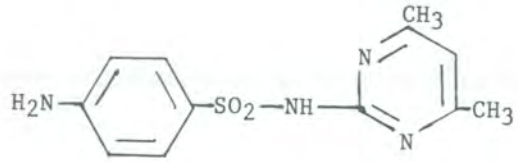
SULFADIMIDINE

IDENTITY

Chemical name: 4-amino-N-(4,6-dimethyl-2-pyrimydyl) sulphanilamide

Synonyms: Sulfamidine, Sulfamezathine

Structural formula:



Molecular formula: $C_{12}H_{14}N_4O_2S$

Molecular weight: 278.32

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient

Melting point: 176°C (depends on crystal structure)

RESIDUES IN ANIMALS AND THEIR EVALUATION

PHARMACOKINETICS

Pigs

The influence of sex on the pharmacokinetics of sulfadimidine (SD) was examined in male, female and castrated male pigs. Sex did not alter significantly elimination rates of SD and its metabolites into urine (Duffee et al, 1984).

SD was fed at the rate of 500 g per metric ton (5 times normal dose) for 30 days to pigs (18-43 kg). When the drug was withdrawn the bedding of the animals was not changed and thus the treated pigs and one group of non-medicated pigs were exposed to contaminated bedding. The contaminated bedding was found to contain SD in amounts from 42-117 ppm.

Mean plasma concentrations of SD were in a linear logarithmic depletion relationship with time with a short half life of 12.6 hours.

After withdrawal of the drug, the concentrations of SD in muscle and fat tissues depleted to <0.1 mg per kg in 4 days and to this level in liver and kidney within 8 - 10 days. Fat and muscle concentrations were 0.1 ppm or less within 4 days at which time the concentrations in the liver were 3.7 ppm and 2.2 ppm, respectively. It was concluded in this study that depletion of tissues and plasma of treated pigs had a linear relationship with time when the concentrations are plotted on a semilogarithmic graph. The values for the half life and time to deplete to 0.1 ppm can be calculated from the graph and are as follows:

Tissue	Half life (hours)	Time to reach 0.1 ppm (days - approx.)
Plasma	12.6	4
Muscle	16.5	4
Liver	31.5	8
Kidney	26.6	7
Fat	16.9	4

The semilogarithmic relationship was maintained over the early part of the withdrawal period but the recycling of the SD from the bedding and the limitations of the method to measure 0.1 ppm or less gave variable results towards the end of the experimental period (Samuelson et al, 1979).

The Food Safety and Inspection Service (FSIS), USDA, carried out a study using 70 pigs (90-113 kg) fed 110 grams SD per ton of feed for 30 days and reduced the level of SD from 1.1 to 13.9 grams per ton for an additional 15 days. The purpose was to determine the serum and tissue concentrations of swine at slaughter when withdrawal feeds were contaminated with various concentrations of SD, to compare serum/liver ratios and the usefulness of serum/tissue ratios in making regulatory decisions on tissue SD concentrations.

There was some difficulty in producing homogenious feeds, although the SD concentrations in all mixed feed batches was within the ± 25% analytical variation permitted. The basal or non-medicated feed fed to control groups was contaminated with a mean concentration of 1.1 gram SD per ton of mixed feed. Nevertheless the feed batches were claimed to be at least as good as those available commercially. Obviously it was not possible to obtain constant dietary intake because of the feed composition variation.

At the beginning of the withdrawal period the pigs were moved to clean pens and this was repeated 24 hours later. This should have reduced the major effect of recycling the excreted residues from the main 30 day treatment period. How this reflects normal pig management in the USA is not stated.

The correlation between serum and tissue concentrations was:

	Slope	Intercept	r (correlation)
Serum/liver	1.37	0.05	0.956
Serum/muscle	3.62	0.07	0.935

However there was large variation at the lower concentrations up to 0.2 ppm.

Concentrations of SD were <0.1 ppm in pigs fed <2 grams SD per ton and <0.1 ppm in muscle and pigs fed <8 grams per ton. When serum SD was <0.1 ppm all liver and muscle samples were also <0.1 ppm. Only when serum SD was >0.45 ppm was it certain that liver SD also exceeded 0.1 ppm. No measurements were made of SMZ in urine (Ashworth et al, 1986).

The excretion of SD into both urine and faeces was determined in a radiometric study using C14-SD administered orally to pigs. The results in table I show that the amount of radioactivity excreted reached plateau values of 72.2% in urine and 16.8% in faeces of the dose of C14 administered. The plateau values were reached at about 6 days after the drug was withdrawn (FDA 224-81-005 & USDA SEA-12-14-3001-064, 1984).

TABLE I. Cumulative excretion of carbon-14 in the urine and faeces (percentage of C14 given to that point in experiment) of pigs dosed with C14 SD

Hours after 1st dose of C14-SD	WT (days)	Urine %	Faeces %	Total %
0-24		37.5	2.4	39.9
0-48		41.5	5.2	46.7
0-96		50.4	8.2	58.6
0-144		54.3	9.8	64.1
0-168	0	59.2	10.5	69.7
0-192	1	66.8	13.6	80.4
0-216	2	69.7	14.6	84.3
0-240	3	70.8	15.6	86.4
0-264	4	71.7	16.2	87.9
0-288	5	72.0	16.4	88.4
0-312	6	72.1	16.6	88.7
0-360	8	72.2	16.7	88.9
0-396	9.5	72.2	16.8	89.0

WT is withdrawal time in the depletion study. Each value is the mean for three pigs.

Data from radiometric study FDA 224-81-005 & USDA SEA-12-14-3001-064. (1984).

Ruminants

Elimination in milk of dairy cattle

Five French Frisonne lactating cows were each administered 100 mg SD sodium salt per kg live weight by intravenous injection. Five similar cows were given three daily intramammary injections of 200 mg SD combined with 40 mg Trimethoprim into each quarter. The injections were carried out after the morning milking and milk was collected at 0800 hours and 1700 hours for a further 7 days. The residues of SD were determined in the milk samples and the results are shown in table II.

TABLE II. Residues of SD in the milk of cows treated parentally with either an intravenous injection of SD sodium salt (100 mg per kg) or after three administrations of 200 mg SD and 40 mg trimethoprim in each quarter

		Residues (ppm)			
		Intravenous treatment		Intramammary treatment	
Withdrawal time (days)	Time of milking	Mean	Maximum	Mean	Maximum
0.4	PM	24.6	31.5	9.97	20.6
1	AM	5.74	6.76	3.04	4.59
	PM	2.26	2.96	13.9	18.1
2	AM	0.55	0.82	0.46	1.0
	PM	0.22	0.36	0.17	0.79
3	AM	0.12	0.20	0.08	0.37
	PM	0.06	0.10	0.02	0.02
4	AM	0.04	0.07	<0.01	0.03
	PM	0.03	0.05	<0.01	0.01
5	AM	0.03	0.05	ND	ND
	PM	0.02	0.03	-	-
6	AM	<0.01	0.01	-	-
	PM	ND	ND		

The mean values are taken for five cows. ND is not detectable (<0.01 ppm).
Residues measured by HPLC method.

After intravenous injection the concentration of SD was highest at the first milking and declined to less than the limit of detection 6 days after treatment. The elimination curve was biexponential.

After intramammary administration the concentrations of SD were highest at the first milking and declined rapidly thereafter to non-detectable levels 5 days after treatment.

In a further experiment on groups of five similar cows SD was administered by intravenous injection (4 and 8 mg per kg) or intramuscular injection (8 mg per kg) in a cocktail of sulfas containing the sodium salts of SD, sulfamerazine and sulfadiazine. The results are shown in table II. At these low doses the concentrations do not exceed 1 ppm for any of the three drugs. Residues of the drugs could be detected after the third milking. No calculations of the % dose excreted into the milk was recorded. (Boisseau, 1988).

Sheep

SD sodium salt was administered intravenously to 14 ewes and the concentrations of SD and its amino substituted metabolites determined by the classical diazotization reactions combined with TLC. The results are shown in table III. 71% original dose was excreted into the urine and excretion was essentially complete 60 hours after dosing. (Bevill et al., 1977a).

Table III: SD concentrations (ppm) in tissues and fluids of sheep after intravenous administration of 107.25 mg SD sodium salt per kg live weight

Hours after dosing	Muscle	Liver	Kidney	Fat	Plasma	Urine % dose accumulated
6	56.8	68.9	124.2	38.9	172	21.6
12	21.2	36.8	63.4	15.0	105	40.1
24	4.10	5.98	18.3	1.70	29	59.7
36	0.98	1.57	5.2	0.35	4	66.4
48	0.15	0.21	0.68	0.9	1	68.4
60	0.13	0.23	0.42	0.03	0	71.0
84	0.09	0.11	0.14	0.02	-	-

The value for tissue residues at 6 hours is for a single animal, all other values are the mean of two sheep.

Poultry

SD elimination in eggs

Two groups of six laying hens were treated with SD sodium salt at a dose of 1 g per L or 2 g per L in their drinking water for 5 consecutive days.

SD was measured by HPLC in both the yellow and white parts of the egg.

The elimination of SD was characterised by significant concentrations in both the white and yellow components. More SD was eliminated in the white which is the inverse of many antibiotics. In the white the concentrations after 1 day were >10 ppm and remained above this level for the duration of the treatment. In the yellow, the concentrations rose progressively but were always less than the levels in the white.

24 hours after drug withdrawal the concentrations in the eggs declined (see Tables IV and V). The elimination was slower in the yellow. About 50% of the dose is eliminated in eggs. (Boisseau, 1988).

TABLE IV: Concentrations of SD in eggs after oral administration for 5 days at the dose of 1 gram SD sodium salt per L in the drinking water

Time (days)	White (ppm) mean	Yellow (ppm) mean	Whole egg (ppm) mean	Whole egg (ppm) maxima
Treatment 1	15.5	3.2	11.6	15.5
2	41.8	14.2	33.0	47.0
3	47.1	25.7	40.1	47.0
4	50.9	28.4	46.0	53.6
5	51.5	34.1	46.0	52.9
Withdrawal 1	34.7	31.6	33.9	41.9
2	4.2	15.6	7.8	11.5
3	2.1	11.7	4.8	6.4
4	1.3	7.5	3.2	3.6
5	0.37	3.2	1.2	1.4
6	0.23	1.6	0.66	1.0
7	0.059	0.27	0.12	0.18
8	0.027	0.050	0.034	0.069
9	0.016	0.029	0.020	0.033
10	0.013	0.022	0.016	0.028
11	0.010	0.018	0.013	0.023
12	0.0051	0.015	0.0082	0.019
13	<0.0005	0.0099	0.0047	0.013
14	<0.0005	0.0050	0.0023	0.018
15	<0.0005	0.0055	0.0017	0.017
16	-	<0.0005	0.0004	0.0019
17	-	<0.0005	-	-

Each value is the mean or maxima of 6 birds which layed 3 - 6 eggs per day.

TABLE V. Concentration of SD in eggs after oral administration for 5 days at the dose of 2 g SD Sodium salt per l in the drinking water

Time (days)	White (ppm) mean	Yellow (ppm) mean	Whole egg (ppm) mean	Whole egg (ppm) maxima
Treatment 1	22.8	4.8	17.0	37.0
2	48.8	18.6	39.4	59.5
3	60.5	33.7	51.9	75.5
4	73.2	38.1	62.6	79.2
5	70.4	44.5	62.1	83.5
Withdrawal 1	43.2	43.8	43.4	54.0
2	7.8	25.3	13.0	17.4
3	2.2	17.3	6.6	7.4
4	1.3	11.3	4.2	4.5
5	1.1	7.6	3.0	4.7
6	0.43	4.1	1.5	1.6
7	0.14	1.0	0.42	0.92
8	0.058	0.35	0.12	0.24
9	0.026	0.025	0.025	0.038
11	0.017	0.012	0.015	0.087
13	0.014	0.0078	0.012	0.018
16	0.010	<0.005	0.0074	0.012
19	0.0061	<0.005	0.0043	0.011
23	<0.005	-	0.0017	0.0053
27	<0.005	-	0.0006	0.0037

Each value is the mean or maxima of 6 birds laying a total of 2 - 6 eggs per day.

RESIDUES

Introduction

Some data on residues was discussed in the section entitled Pharmacokinetics. This section expands the information on metabolism and discusses the information on residues reported in six studies.

Metabolism

In farm animals, rodents and humans, there is acetylation of the aromatic amino group in the reticuloendothelial cells of the liver and in other tissues to yeild N-acetyl SD, a major metabolite of SD. Two other metabolites of SD in pigs were identified in the radiometric study, namely, N-glucose SD and desamino SD. The production of desamino SD probably occurs in cattle, sheep and poultry but is not thought to be of importance in humans. A complication is that residues of SD in liver and muscle of pigs are converted either chemically or enzymatically to N-glucose-SD during storage at -20°C. Thus the ratio of SD to N-glucose-SD in pig meat will depend to some extent on how long the meat is stored before eating.

The small amounts (<5% of total residues) of polar metabolites have not been identified in most studies, but it is thought that they are produced by the hydroxylation of the aromatic ring which may be further conjugated with glucuronic acid. (FDA 224-81-005 USDA SEA-12-14-3001-064, 1984; Bevill et al., 1977a; Baggot, 1983; Wooley & Siegel, 1982; Kietzmann, 1981).

Residues in farm animals and tissues

The data from six studies are given in the tables as follows:

Table No.	Source	Species/ product	Route (dose)	Analytical method
II	Boisseau, France	milk	i.v. (100 mg/kg) or i.m. (200 mg/ quarter)	HPLC
III	Bevill et al, USA	sheep	i.v. (107.25 mg/kg)	Colorimetric
IV, V	Boisseau, France	eggs	oral in water (1-2G/L)	HPLC
VI	Ashworth, USA	pig	oral (110g/ton feed)	TLC
VII	Samuelson, USA	pig	oral (550 g/ton feed)	Colorimetric
VIII, IX	FDA, USDA, USA	pig	oral (110/ton feed)	C-14

Tables VI and VII indicate total residues of SD, measured by chemical means, in various tissues from pigs. In table VI the pigs were fed 110 g SD per ton of feed daily for 30 days and then placed on a ration containing 1-14 g SD per ton for 15 days before slaughter. In table VII the pigs were fed at 550 g SD per ton of feed daily for 30 days and then slaughtered after a withdrawal period varying from 0 to 12 days. At 12 days no detectable residue remained.

TABLE VI: Residues of SD in muscle and liver of five pigs fed 110 grams per ton feed daily for 30 days and then fed SD at different daily levels for 15 days before slaughter

SD in withdrawal feed (g per ton)	Residues (ppm) Muscle		Liver	
	mean	maximum	mean	maximum
1.1	0.008	0.014	0.04	0.06
2.03	0.015	0.026	0.10	0.19
2.36	0.014	0.030	0.13	0.18
5.74	0.074	0.10	0.34	0.46
13.5	0.14	0.16	0.60	0.74
13.9	0.16	0.22	0.68	0.79

Residues measured by TLC-fluorescence method of Thomas et al.; (1981).

TABLE VII: Residues of SD in tissues of pigs fed 550 grams per metric ton feed for 30 days

Withdrawal period (days)	Residues (ppm) muscle	liver	kidney	fat
0	5.77 (0.37)	18.27 (0.91)	16.07 (0.46)	4.90 (0.30)
2	0.67 (0.16)	2.81 80.40)	1.86 (0.46)	0.43 (0.12)
4	0.09 (0.02)	0.37 (0.1)	0.22 (0.07)	0.10 (0.02)
6	0.02 (0.00)	0.05 (0.03)	0.16 (0.06)	0.02 (0.01)
8	0.02 (0.01)	0.12 (0.08)	0.08 (0.05)	0.01 (0.01)
10	0.00	0.06 (0.02)	0.01 (0.01)	0.00
12	0.00	0.00	0.00	0.00

Each value is the mean value for 3 pigs; the values in parentheses are the SEM. Residues measured in plasma and feed by adaption of method of Annino, (1961), and in tissues by method of Tishler, (1968).

The radiometric study gives the only complete information on total residues because all of the other analytical methods miss one or more of the metabolites. The radiometric study will be discussed in detail because of its importance.

RADIOMETRIC STUDY IN PIGS

Summary

Pigs were administered feed containing 110 g SD per ton and 76 uCi Carbon-14 labelled SD (C14-SD) in two studies, a steady state residue experiment and a residue depletion experiment. The nature and quantity of residues in tissues and excreta were measured using combinations of radiometry with modern methods of separation and identification.

The results show that SD is converted in the live pig to three major metabolites, N-acetyl-SD, N-glucose-SD and desamino-SD. SD is also converted to N-glucose-SD in stored deep frozen liver and muscle tissues.

The major residues in all tissues were a mixture of parent drug and the three metabolites. The concentrations of residues were highest in the blood, GI tract plus contents, liver and kidney and depleted in all tissues to levels below 0.1ppm 10 days after drug withdrawal (see table VIII).

TABLE VIII: Concentration (ppm SD equivalents) of C14-residues in tissues of pigs dosed with C14-SD

Tissue	8 hr depletion animal number			2 day depletion animal number		
	10	11	12	13	14	15
Blood	11.36	10.01	13.85	2.61	4.67	4.59
Kidney	9.40	7.78	10.64	2.20	2.95	3.29
Skeletal muscle	3.24	2.82	3.40	0.74	0.98	1.07
Liver	6.52	6.29	8.11	1.59	2.82	2.81
Fat	1.16	1.10	1.34	0.26	0.37	0.38
Brain	2.62	2.39	3.11	0.65	0.86	0.93
Heart	4.84	4.78	5.94	1.23	1.81	1.82
Spleen	3.35	3.48	4.24	0.82	1.24	1.29
Lung	4.65	4.78	6.47	1.29	1.77	2.02
GI tract + contents	13.02	11.22	15.96	5.88	8.40	8.50

Tissue	5 day depletion animal number			10 day depletion animal number		
	16	17	18	19	20	21
Blood	0.44	0.10	0.28	0.027	0.037	0.019
Kidney	0.28	0.08	0.19	0.021	0.033	0.024
Skeletal muscle	0.10	0.02	0.05	0.004	0.005	0.003
Liver	0.32	0.15	0.21	0.048	0.071	0.066
Fat	0.04	0.01	0.02	0.003	0.004	0.002
Brain	0.09	0.02	0.04	0.003	0.005	0.003
Heart	0.23	0.03	0.09	0.011	0.014	0.013
Spleen	0.14	0.04	0.09	0.018	0.016	0.015
Lung	0.20	0.05	0.12	0.017	0.027	0.017
GI tract + contents	0.82	0.34	0.55	0.030	0.070	0.06

Note: All animals were dosed with C14-SD at 12 hour intervals for 7 consecutive days. The animals were sacrificed at 8 hours, 2 days, 5 days or 10 days after the last dose.

Animals and treatments

Steady state experiment - Five gilts and five barrows weighing 47-67 kg were fed at 12 hour intervals for 3, 5 or 7 days with a 1.5 kg ration containing 165 mg SD and 75.94 uCi C14-SD. Urine and faeces were collected separately at 24 hour intervals after the initial dose, 12 hours after the penultimate dose and 8 hours after the last dose. All the animals were sacrificed 8 hours after the last dose and tissue samples collected and stored at -20°C until analysis.

Residue Depletion Experiment - Twelve pigs were dosed orally with C14-SD for 7 consecutive days as described for the steady state experiment. The pigs were slaughtered in groups of three at 8 hours, 2, 5 and 10 days after withdrawal of the drug. Total urine and faeces were collected at 24 hour intervals throughout the dosing and depletion periods of the experiment. Tissue samples were collected and deep frozen at -20°C until analysed.

Methods

The total radioactivity of C14 was determined in aliquots of fluids, homogenised tissues and faeces. The activity in urine and fat was measured in solutions of the samples in liquid scintillator, whereas other tissues were measured after combustion of the C14-metabolites to C14-CO2. Only about 90% of the activity was recovered in the combustion method because the aromatic ring of SD limited total combustion.

The metabolic products of SD were isolated by a combination of solvent/solvent partition, XAD-2 column chromatography and finally HPLC. The products were divided essentially into those which were extracted into either methanol, hexane or water. More than 80% of the total C14-activity was extracted from muscle, liver and kidney samples into methanol in the steady state experiment or after 8 hours withdrawal time in the depletion experiment, however as withdrawal time increased through 2, 5 and 10 days there was a gradual rise (>70% in the liver) in the percentage of C14-activity that was not extracted into methanol. 39-59% of the activity was extracted into methanol from fat. The hexane extract always contained <22% of the total residues (see table IX).

The metabolites were identified from the HPLC fractions by using specific ion monitoring of GC-MS and electron-impact mass spectrometry of the N-Methyl derivatives. C14-labelled standards were prepared for the 3 metabolites. These were used for spiking control tissues and then confirming the methods.

TABLE IX: The distribution of carbon-14 in methanolic extracts of tissues of swine after withdrawal of C14-SD

Withdrawal time & tissue	C14 total ppm	C14 in XAD2/MeOH extract	% C14-metabolite in MeOH extract			
			SD%	Acetyl -SD %	Glucose -SD%	Desa-mino -SD%
Muscle						
8 hours	3.2	80.5	44.0	8.2	29.1	2.2
2 days	0.93	80.7	41.0	8.3	39.8	2.3
5 days	0.06	71.8	47.4	7.8	31.7	4.7
10 days	0.004	62.1	23.8	4.5	11.4	48.7
Liver						
8 hours	7.0	83.1	30.6	17.9	43.9	1.8
2 days	2.4	74.0	28.4	12.7	51.0	2.4
5 days	0.23	56.5	31.8	6.9	48.2	6.0
10 days	0.06	24.0	31.3	10.9	22.1	24.7
Kidney						
8 hours	9.3	91.2	50.5	30.4	5.5	6.7
2 days	2.8	89.2	53.8	28.1	5.2	4.2
5 days	0.18	73.5	54.8	25.1	5.0	7.4
10 days	0.03	35.3	26.6	13.3	6.7	42.4
Fat						
8 hours	1.2	58.5	41.1	18.6	15.4	11.1
2 days	0.38	50.6	45.2	17.9	7.2	13.2
5 days	0.02	39.1	30.3	14.5	10.3	22.0
10 days	0.003	40.6	11.9	3.7	0.1	75.1

Each value is the mean for three pigs.

The maximum mean concentrations for the metabolites was observed in the tissues collected at 8 hours withdrawal time. They were: SD, 4.3 ppm in kidney; Acetyl-SD, 26 ppm in kidney; N-Glucose-SD, 2.6 ppm in liver; Desamino-SD, 0.56 ppm in kidney.

Results

The results for the pigs fed for 7 days and slaughtered after 8 hours in the steady state experiment are similar to those found for the pigs slaughtered after 8 hours withdrawal time in the depletion experiment.

Metabolites

The four major compounds in the residues were identified as

1. Parent drug-SD
2. N-acetyl-SD
3. N-glucose-SD
4. Desamino-SD

SD was the major residue in blood, liver, muscle, kidney and fat from all animals in the steady state experiment. The major residues found in liver tissues were near equal amounts of SD and N-Glucose-SD. However it was proposed that SD is readily converted to N-Glucose-SD during storage of frozen tissues and so SD was probably the most abundant residue at the time of slaughter.

In the depletion kinetics study (table VII) there was a substantial increase in the percentage of the total C14-activity in all the tissues present as Desamino-SD as the withdrawal time increased through 2, 5 and 10 days. The percentage of total activity attributable to SD, N-acetyl-SD and N-glucose-SD decreased as the withdrawal time extended. However the total concentrations of residues at 10 days withdrawal time was <0.06 ppm and thus the actual amount of Desamino-SD was very low but it is the most persistant residue.

Total residues

The total residues of C14 expressed as ppm SD are shown in table IX. The highest concentration of total residues (16.0 ppm) was found in the GI tract and its contents, the maximum concentrations in blood (13.9 ppm), kidney (10.6 ppm) and liver (8.1 ppm) were also high whereas the maximum concentrations in muscle (3.4 ppm), brain (3.1 ppm) and fat (1.3 ppm) were the lowest in the tissues examined. The concentrations in the GI tract and its contents remained the highest at all withdrawal times but the maximum concentrations seen in any of the tissues was 4.6 ppm in blood after 2 days withdrawal, 0.44 ppm in blood after 5 days withdrawal time and 0.048 ppm in kidney after 10 days withdrawal time. Many of these very low residues at 5 and 10 days withdrawal time would have been too low to be measured by most of the non-radioisotopic analytical methods.

The total residues in four tissues and blood are plotted semilogarithmically against withdrawal time in Figure 1. This shows how the concentrations of residues in the tissues decrease. The pattern of depletion is not dissimilar from that observed in other studies. Certainly after 10 days the residues are very low. (FDA 224-81-005; USDA SEA-12-14-3001-064, 1984).

APPRAISAL

The depletion study provides the most important information on residues of SD fed the drug under conditions similar to the proposed use in pig production. N-glucose-SD and desamino-SD are two important metabolites which are not reported in the other studies.

The other two studies using oral feeding of SD to pigs (tables VI and VII) encountered problems with both coprophagia and cross-contamination of feeds with SD during the mixing and mill processing of the feeds.

The maximum residues in pigs fed SD are in the range 5-30 ppm and were found in blood, liver and kidney. The residues remain highest in these tissues throughout the first few days of a withdrawal period. This is well illustrated in the depletion curves shown in figure 1.

In all the studies where SD was dosed orally the residues decline to very low levels at some time during the second week of the withdrawal period. After intravenous injection or intramammary infusion of SD into lactating cows the concentrations of residues in milk declines very rapidly from maximum values of 21 and 32 ppm to <0.05 ppm after 5 days withdrawal.

Many nations have set tolerances for residues of SD with the most common being 0.1 ppm or zero. However, it is not always clear whether these tolerances are set for total residues or for the residue of the parent drug. In practice the residue of the parent drug is the compound normally monitored.

In the six studies presented, only the radiometric study gives information on total residues, threee studies report levels of parent drug and two studies report

concentrations of parent drug plus those metabolites which have the amino group attached to aromatic ring and can therefore give positive coupling reactions in the diazotization reaction. Only the radiometric study measures the residues of desamino-metabolites. The studies are summarised in table X.

TABLE X: Summary guide to residue studies using SD

Species	Tissue	Source Table no	Residue measured	Analysis
Cattle	milk	II	SD	HPLC
Sheep	M,L,K,F,P	III	SD + Ac-SD + other amine-derives	Diazo
Poultry	Eggs	IV, V	SD	HPLC
Pigs	M,L	VI	SD	TLC-fluor
Pigs	M,L,K,F	VII	SD + Ac-SD + other amine-derives	Diazo
Pigs	M,L,K,F,B + others	VIII	Total SD residues	C14
Pigs	M,L,K,F	IX	SD, Ac-SD, Glucose-SD Desamino-SD	C14

M is muscle, L is liver, K is kidney, F is fat, P is plasma, B is blood, U is urine. The methods are Diazo = Diazotization; TLC-Fluor = TLC and fluorescence. C14 is the radiometric assay after using C14-SD; HPLC is from study by Boisseau (1988).

The residues are vey high during and immediately after drug administration and a withdrawal time seems essential. Once a tolerance is decided the six studies provide a substantial amount of information which might be used to establish recommended withdrawal times.

The relationship between total concentrations of residues of SD and the concentration of parent drug is calculated from the data provided in the radiometric study and is shown in table XI. This data is a clear indication that, at least in the pig, residues of parent drug at levels of 0.1 ppm or higher are about one fifth to one half the total residues in edible tissues and at concentrations below 0.1 ppm the parent drug may account for as little as 5% total residues.

The pharmacokinetic studies show there is reasonable certainty that monitoring SD concentrations in body fluids can be used to indicate likely concentrations of SD in tissues. This method of control is practised in some countries and early reports show that is has been instrumental in reducing the number of violations in the USA pig industry.

FIGURE 1

RESIDUES OF C14-SD IN PIGS

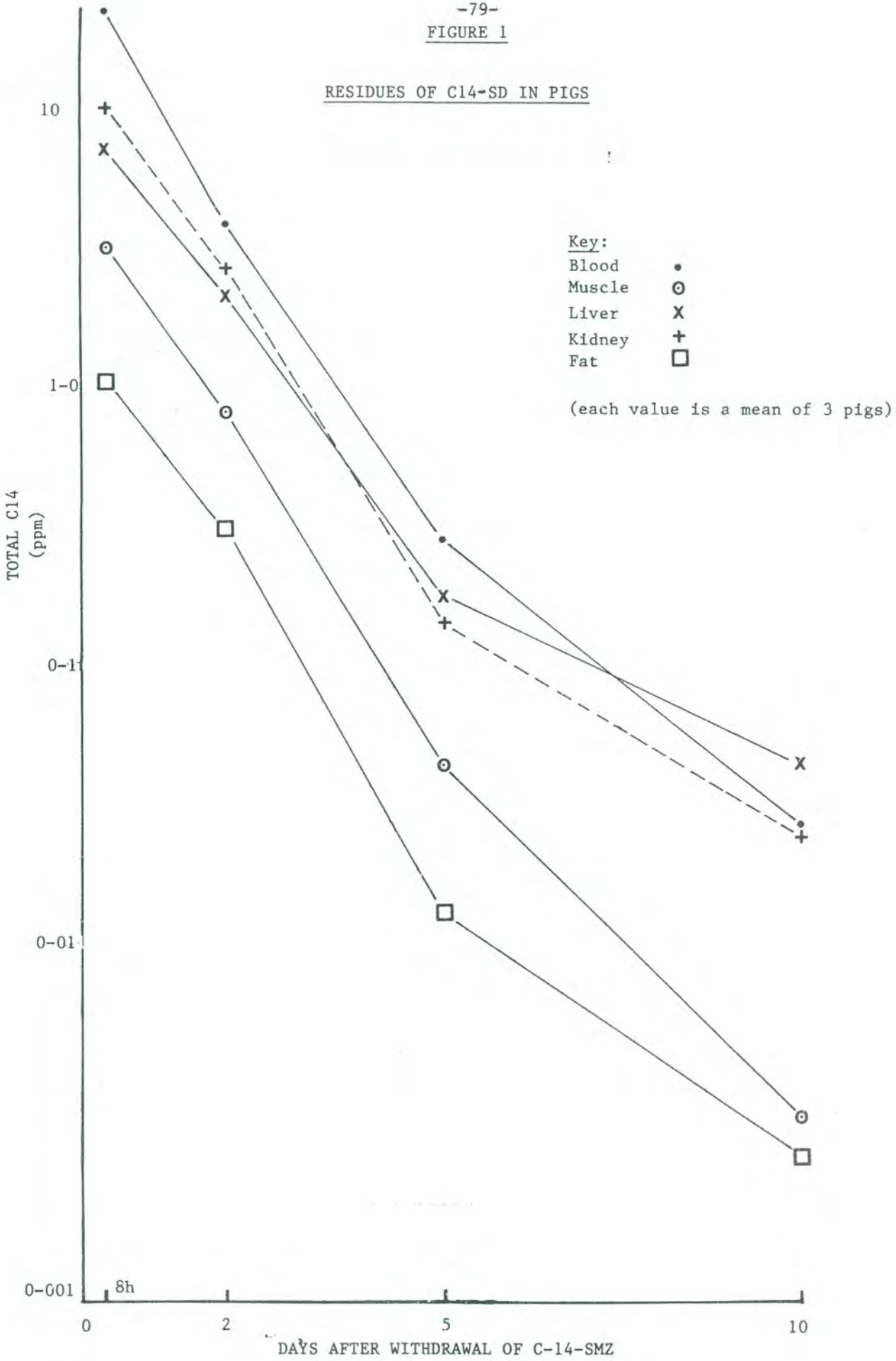


TABLE XI: Comparison of SD residues and total residues in pigs after withdrawal of feed containing C14-SD

Withdrawal time & tissue	C14 total ppm	SMZ ppm	SMZ % total
Muscle			
8 hours	3.2	1.13	35
2 days	0.93	0.31	33
5 days	0.06	0.02	34
10 days	0.004	0.0006	15
Liver			
8 hours	7.0	1.78	25
2 days	2.4	0.50	21
5 days	0.23	0.04	18
10 days	0.06	0.004	8
Kidney			
8 hours	9.3	4.38	46
2 days	2.8	1.34	48
5 days	0.18	0.07	40
10 days	0.03	0.003	10
Fat			
8 hours	1.2	0.29	24
2 days	0.38	0.09	23
5 days	0.02	0.002	12
10 days	0.003	0.0001	5

Each value is the mean for three pigs.

REFERENCES

Annino J.S., (1961), Sulphonamides, in Standard Methods of Clinical Chemistry, Ed by Sligson D.E., New York, Acad. press, vol. 3, pp 200-206.

Ashworth, R.B., Epstein, R.L., Thomas, M.H., and Frobish, L.T. (1986) Am. J. Vet. Res., 47, 2596-2603.

Baggot, (1983), "Systemic antimicrobial therapy in larger animals". In "Pharmacological Basis of Large Animal Medicine", Eds. Bogan, J.A., Lees, P. and Yoxall, A.T., Blackwell Scientific Publ. 45-69.

Bevill, R.F., Sharma, R.M., Meachum, S.H., Wozniak, S.C., Bourne, D.W.A. and Dittert, L.W. (1977a), Am. J. Vet. Res. 38, 973-977.

Boisseau, J., private communication (1988).

Duffee, N.E., Bevill, R.F., Thurmon, J.C., Luther, H.G., Nelson, D.E. and Hacker, F.E., (1984), J. Vet. Pharm. Ther. 7, 203-211.

FDA & USDA (1984), The Fate of Carbon-14 given to Swine as 14C-Sulfamethazine. USDA, SEA-12-14-3001-064, FDA-224-81-005.

Kietzmann, M. (1981), Arch. Geflugelkd. 45, 233-239.

Samuelson, G., Whipple, D.M., Showalter, D.H. Jacobson, W.C. and Heath, G.E. (1979) J. Am. Vet. Med. Ass, 175, 449-452.

Thomas, M.H., Soroka, K.E. and Simpson, R.M., (1981), J. Agric, Food Chem., 29, 621-628.

Tishler, F., Sutter, J.L., Bathish, J.N. and Hagman, H.E., (1968) J. Agr. Food Chem., 16, 50-53.

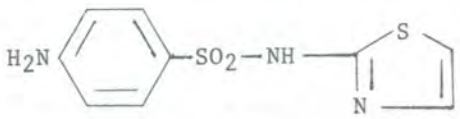
Wooley, J.L. and Siegel, C.W. (1982), Am J. Vet. Res. 43, 768-774.

SULFATHIAZOLE

IDENTITY

Chemical name: 4-amino-N-2-thiazolylbenzenesulfonamide

Structural formula:



Molecular formula: $C_9H_9N_3O_2S_2$

Molecular weight: 255.32

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient

Melting point 200-204°C

RESIDUES IN ANIMALS AND THEIR EVALUATION

PHARMACOKINETICS

Pigs

The pharmacokinetics of sulfathiazole (STZ) in swine following both parental and oral administration of the drug have been well investigated. The data obtained fit simple pharmacokinetics models. These models permit prediction of residue depletion.

Pigs - Parental Administration

Pigs (33-40kg) were injected into the femoral vein with a single dose of a 12.5% solution of sodium salt of STZ equivalent to 72mg per kg live weight. Blood and urine samples were collected for 25 hours and analysed for residues of STZ. The biological half-life was 1.4 hours and the volume of distribution was 0.54 L/kg. Within 12 hours of administration the STZ in plasma was no longer detectable and the excretion of residues into the urine was complete.

The amount of residues in the urine as a percentage of the original dose were:

STZ 60%; Acetyl-STZ 23%; Polar metabolites 3%

Thus about 86% of the dose was excreted into the urine within 12 hours and the remainder was excreted by an extrarenal route (Koritz et al, 1978).

In a further study by the same group, pigs (32-36 kg) were administered the same dose as above and slaughtered 2-24 hours after dosing. The half-life of STZ was 1.6 hours and the volume of distribution 0.625 L/kg.

The cumulative excretion of residues into urine again reached plateau values about 12 hours after dosing with STZ = 48% dose; and the acetyl-STZ = 19% original dose.

Concentrations of STZ were measured in muscle, liver, kidney and fat and the data for all tissues was closely correlated ($r > 0.994$ see table I) with the plasma concentration and the rate of excretion into the urine. This indicates that STZ rapidly penetrates and

equilibrates with a large number of extravascular tissues. The authors concluded that using simple pharmacokinetic models the tissue residues can be estimated with accuracy from plasma and/or urine concentrations of the drug and a valid estimate can be made of the time required for tissue residue depletion to tolerance without slaughtering the animals (Bourne et al, 1978).

Table I: STZ concentrations in tissues and fluids of pigs after intravenous administration of 72 mg STZ-Na per kg live weight

Hours after dosing	Muscle (ppm)	Liver (ppm)	Kidney (ppm)	Fat (ppm)	Plasma (ppm)	Excretion rate (% dose/hr)
2	24.8 (2.8)	37.5 (2.2)	147 (51.3)	9.1 (1.6)	44.9	9.37
4	7.9 (1.6)	15.6 (2.9)	46.5 (7.7)	6.2 (1.9)	18.9	4.58
8	1.2 (0.2)	2.9 (0.3)	7.7 (0.8)	0.5 (0.1)	4.4	1.14
16	0.2 (0.15)	0.3 (0.12)	1.3 (0.3)	n.s.	0.8	0.11
24	0.15 (0.1)	n.s.	n.s.	n.s.		
r between plasma and tissues	0.996	1.000	0.994	0.940		
r between excretion rate and tissues	0.986	0.997	0.985	0.961		

Each value is the mean of three animals with the standard deviation in parentheses.
n.s. indicates the means are not significantly different ($p < 0.01$) from the mean value for three control untreated pigs.

r is the correlation coefficient.

Residues measured by method of Annino, (1961).

Pigs - Oral Administration

Pigs (33-40 kg) were given a single capsule by gastric intubation of a solution of sodium salt of STZ equivalent to 214 mg per kg live weight. Plasma concentrations of STZ and urinary concentrations of residues were measured over a 24 hour period after dosing. The half-life of absorption was 0.8 hours and about 73% of the original dose was excreted in the urine within 24 hours. There was almost 3 times as much parent STZ as the acetyl-STZ in the urine with only a few percent of the residues excreted as polar metabolites.

Thus the sodium salt of STZ is rapidly absorbed from the gut and is rapidly excreted undergoing some metabolism into the urine. (Koritz et al, 1978).

In an earlier study 16 pigs (43-53 kg) were given sodium-STZ in capsule form at a therapeutic level (330 mg per kg live weight) for 3 days. A 12 hour dosing schedule was used. The pigs were slaughtered at regular intervals over a 12 day withdrawal period. Tissue and urine samples were collected and analysed for residues of STZ. The results are shown in Table II.

The concentrations of STZ were very high in all tissues and urine of the pigs slaughtered within 12 hours of drug withdrawal. Thereafter there was a rapid decline in the residues

such that although residues were detected at day 7 no residues in the tissues were found at the next sampling points, days 10 and 12 (Righter et al, 1971).

Table II. Residues of sulfathiazole in pig tissues and urine after three daily oral doses of sodium-STZ at 330 mg per kg live weight

Withdrawal period (days)	N	Residues (ppm) Muscle	Liver	Kidney	Fat	Urine
0.5	2	2.8 (5.6)	17 (20)	60 (74)	3.0 (3.7)	1405*
3	2	0.06 (0.12)	0.06 (0.11)	0.04 (0.05)	0.1 (0.17)	87.3*
5	3	0.03 (0.07)	0.01 (0.04)	0.06*	0.06 (0.07)	33.2*
7	3	0.11 (0.12)	0.02 (0.04)	0.04 (0.04)	0.13 (0.14)	-
10	3	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.0 (12.2)
12	3	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.000)	-

All values are means except those marked* which are results for only one pig.
N is number of pigs.
The values in parentheses are the maximum values.
Residues determined by method of Annino, (1961).

Ruminants

The pharmacokinetics of STZ were studied in four sheep (ewe-lambs 27--32 kg). STZ was administered as a 5% solution of the sodium salt into the jugular vein and orally as a 12.5% aqueous solution of the sodium salt. The doses were either 36 or 72 mg per kg for the i.v. injections or 214 mg per kg oral intubation.

Blood and urine were collected over 48 hour period for the i.v. dose and 144 hour period for oral dose.

Plasma and urine data were examined. The pharmacokinetics can be described by a one compartmental model. The semi-log plot of the average concentrations of STZ in plasma and also the amount of unchanged STZ excreted into the urine were linear with time in both the i.v. treatments and after 4-6 hours in the oral treatments.

STZ was rapidly eliminated with a biological half-life of 1.3 hours. After oral administration it was absorbed slowly (half-life 18 hours) and 73% was excreted in the urine. Following oral administration the disappearance of STZ from the plasma was controlled by the rate of drug absorption, rather than by elimination.

The major components in the urine were unchanged STZ and the acetyl derivative. STZ was 5-9 times more abundant than Acetyl-STZ. Polar metabolites were also measured and usually made up <5% total residues (Koritz et al, 1977).

The plasma, tissue and urine STZ concentrations were determined at various times following intravenous administration of STZ-Na (72 mg per kg) to 12 female sheep.

The plasma and urine data were consistent with a one-compartment pharmacokinetic model, with an elimination half life of 1.1 hour. After 10 hours 87% of the original dose, wax excreted into the urine. 67% original dose was STZ, 18% was the acetyl-STZ and about 2% was an unidentified polar metabolite.

The data from the tissue sites (see table III) also were consistent with the one-compartment model and confirmed that tissue residues of STZ can be calculated from urine and plasma concentrations. The correlation coefficients (see table III) for the concentrations between the fluids and the tissues all exceeded 0.994 (Bevill et al., 1977 b).

Table III. STZ concentrations in tissues and fluids of sheep after intravenous administration of 72 mg STZ-Na per kg live weight

Hours after dosing	Muscle (ppm)	Liver (ppm)	Kidney (ppm)	Fat (ppm)	Plasma (ppm)	Excretion rate (% dose/hr)
2	22 (5.7)	40 (13)	308 (145)	11 (5.2)	47	13.4
4	4.9 (2.8)	9.4 (4.9)	55 (24)	3.5 1.9)	12	4.3
8	0.23 (0.11)	0.70 (0.29)	2.3 (1.3)	0.26 (0.13)	1	0.7
16	0.05 (0.02)	0.30 (0.14)	0.36 (0.13)	n.s.		
24	n.s.	0.12	0.11	n.s.		
r between plasma and tissues	0.999	1.000	1.000		0.998	
r between excretion rate and tissues	0.995	0.996	0.990		1.000	

Each value is the mean of three animals with the standard deviation in parentheses.
n.s. indicates that the means are not significantly different ($p < 0.01$) from the mean value for three control untreated sheep.
r is the correlation coefficient.
Residues measured by method of Bevill et al (1977a).

Humans

The kinetics of excretion into urine and acetylation were studied in normal adult humans. Excretion of free STZ, it's acetylation, and excretion of acetyl-STZ were described by a kinetic model previously shown to hold for the same processes in the disposition of sulfisoxazole and sulfamethylthiadazole when 0.5 gram doses were ingested. The disposition of these drugs by the human consists of two competitive first-order processes, one for acetylation and the other for excretion of the free drug.

Normal adults were given oral doses (0.5 - 1 g) of STZ and the amounts of STZ and acetyl-STZ measured in regular urine samples collected over a 36 hour period. The cumulative amounts of drug after 36 hours are shown below:

Dose (mg)	STZ (mg)	Acet-STZ (mg)	Total mg	% Dose
1000	800	232	1032	103
1000	786	213	999	100
500	408	67	475	95
500	451	79	530	106
500	452	79	531	106
500	371	141	512	102

Acetyl-STZ is calculated as the free drug.

The experimental data fitted the theoretical predictions escept in the first subject receiving a one gram dose. This was because the intake of water was insufficient to maintain a urine flow rate high enough to prevent crystalluria. When the experiment was repeated at a higher water intake the model was obeyed. At a 1 gram dose it was calculated that urine excretion rate must exceed 200 ml per hour to maintain solubility of the acetyl-STZ (Nelson, 1961).

RESIDUES

Metabolism of STZ

There are numerous reports that the major metabolite of STZ in man, rats, pigs, sheep, cattle and rabbits is the N-acetyl derivative.

There is evidence of a small percentage of polar metabolites formed in the pig, sheep and human. Willians (1959) suggests that the polar metabolite in humans is produced by the hydroxylations of the 2-position on the aromatic ring.

Appraisal

The results of two studies in pigs and one in sheep are shown in tables I, II and III. Unfortunately there does not seem to be a definitive radiometric study and only one study where STZ is administered orally in pig feed.

Residues in tissues of pigs (see table II) fed 330 mg STZ per kg live weight were highest in liver (maximum 20 ppm) and kidney (maximum 74 ppm) 12 hours after withdrawal of the drug. The concentrations declined very quickly and in all tissues were <0.18 ppm 3 days after withdrawal and only detectable in one muscle sample (0.01 ppm) after 10 days withdrawal time.

The residues following large intravenous injections of STZ into both pigs (table I) and sheep (table III) produced very high residues for the first few hours after administration but then the drug was rapidly excreted and the residues were ca. 0-0.3 ppm 24 hours after dosing.

REFERENCES

Annino J.S., (1961), Sulphonamides, in Standard Methods of Clinical Chemistry, Ed by Sligson D.E., New York, Acad. press, vol. 3, pp 200-206.

Bevill, R.F., Sharma, R.M. Meachum, S.H. Wozniak, S.C. Bourne, D.W.A. and Dittert, L.W. (1977a), Am. J. Vet. Res. 38; 973-977.

Bevill R.F., Koritz, G.D., Dittert, L.W. and Bourne, D.W.A. (1977b) J. Pharm. Sci. 66; 1297-1300.

Bourne, D.W.A., Dittert, L.W. Koritz, G.D. and Bevill, R.F. (1978), J. Pharmacokinetics Biopharm. 6; 123-134.

Koritz, G.D., Bourne, D.W.A., Dittert, L.W. and Bevill, R.F. (1977) Am. J. Vet. Res. 38; 979-982.

Koritz, G.D., Bevill, R.F., Bourne, D.W.A., and Dittert, L.W., (1978), Am J. Vet. Res. 39; 481-484.

Nelson, E., (1961), J. Pharm. Sci, 50, 912-915.

Righter, H.F., Worthington, J.M. and Mercer, H.D., (1971), J. Anim. Sci. 33; 797-798.

Williams, R.T. (1959), In "Detoxification Mechanisms", John Wiley & Sons, Inc., New York, p. 509.

TRENBOLONE ACETATE

IDENTITY

Chemical name:

Trenbolone

17β-hydroxyestra-4,9,11-trien-3-one
4,9,11-estratrien-17β-ol-3-one
17β-hydroxy-19-norandrosta-4,9,11-trien-3-one
19-norandrosta-4,9,11-trien-17β-ol-3-one

Trenbolone acetate

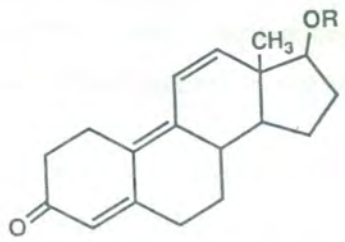
17β-acetoxy-3-oxoestra-4,9,11-triene
17β-acetoxyestra-4,9,11-triene-3-one
3-oxo-17β-hydroxy-4,9,11-estratrieneacetate

Synonyms:

Trenbolone

trienbolone
trienolone

Structural formula:



R = H (trenbolone)
R = $COCH_3$ (trenbolone acetate)

Molecular formula: $C_{18}H_{22}O_2$ (trenbolone), $C_{20}H_{24}O_3$ (trenbolone acetate)

Molecular weight: 270.38 (trenbolone), 312.39 (trenbolone acetate)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient

	Trenbolone	Trenbolone acetate
Appearance:	Pale yellow, crystals	crystals
Melting point:	183 - 186°C	96 - 97°C
Optical rotation:	$[\alpha]_D^{20}$ = +19° (c=0.45 in ethanol)	+36.8° (c=0.37 in methanol)
UV_{max}:	239, 340.5 nm	

(Windholz, 1983)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Trenbolone acetate (TBA) is a synthetic steroid with anabolic properties. It is administered as a subcutaneous implant to the base of the ear and is used to improve body weight, feed conversion and nitrogen retention in cattle 60-90 days or more before the intended date of slaughter. It is used alone or in combination with another hormonally active ingredient. The ear, along with any residual drug, is discarded at slaughter.

Trenbolone acetate upon entering the circulatory system is rapidly hydrolyzed to its free active form, trenbolone (TBOH). In the rat the 17β-epimer is the major metabolite. In the bovine species the 17α-epimer is the major metabolite occurring in the excreta, bile and liver; the 17β-epimer is the major metabolite occurring in muscle. (Jouquey, et al., 1983).

This monograph will explore the residues of trenbolone acetate when used in combination with estradiol and other estradiol-containing products to determine if an increase in either 17β-trenbolone (17β-TBOH) or 17α-trenbolone (17α-TBOH) levels is produced. This determination will be based on three studies in steers, 2 studies in heifers and 1 study in calves.

Dosages

Finaplix (300 mg TBA) = heifers
Torelor (200 mg TBA + 40 mg estradiol-17β) = steers
Revalor (140 mg TBA + 20 mg estradiol-17β) = calves

RESIDUE STUDIES

Steers

Five groups of steers, 6 per group, were used in this study. Group I served as untreated controls. The 24 steers in groups II-V were implanted with Torelor which is 200 mg of trenbolone acetate (TBA) + 40 mg of Estradiol-17β (E2β). One group was slaughtered at each of the following times after implantation: 15 days, 30 days, 60 days, and 75 days. Control steers were slaughtered at each of these time points.

Free and conjugated 17β-TBOH, free and conjugated 17α-TBOH, and free and conjugated estradiol-17β were determined in muscle, liver, kidney, fat, and plasma. Free estrone was measured in liver and fat and conjugated 17α-TBOH and conjugated E2α were determined in urine. Analyses were done by an HPLC-RIA method.

Tissue residues of free and conjugated 17β- and 17α-TBOH are given in Tables I-IV: Levels reported without ranges are at or below the detection limit of the method.

The detection limit was considered to be 70 ng/kg and was defined as the residue level which could be reliably determined (although considerably lower levels are detectable using the analysis method).

TABLE I. Free 17β-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	254 ± 62	467 ± 162	78 ± 41	392 ± 147
30-Day	272 ± 80	323 ± 131	67	293 ± 171
60-Day	108 ± 29	180 ± 105	78 ± 24	120 ± 106
75-Day	71 ± 32	83 ± 52	52	111 ± 86

TABLE II. Conjugated 17β-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	66	1110 ± 568	35	27
30-Day	43	772 ± 618	36	31
60-Day	38	695 ± 337	33	32
75-Day	43	401 ± 177	33	20

TABLE III. Free 17α-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	0	213 ± 71	95 ± 44	74 ± 20
30-Day	9	226 ± 80	76 ± 8	62 ± 19
60-Day	41	89 ± 26	24	60
75-Day	40	39	23	55

TABLE IV. Conjugated 17α-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	21	1918 ± 864	386 ± 282	59
30-Day	10	1708 ± 758	210 ± 44	36
60-Day	27	908 ± 664	143 ± 27	52
75-Day	16	656 ± 331	182 ± 51	16

The concentration of free 17β-TBOH in muscle, liver, and fat are comparable to each other, while in the kidney the concentration is down near the detection limit. Detectable levels of conjugated 17β-TBOH were found only in liver.

Free 17α-TBOH could only be detected in liver until 60 days after implantation and in kidney and fat until 30 days after implantation. Detectable amounts of conjugated 17α-TBOH were found in liver and kidney [(Arts, et al., 1986 (a)].

In a second study, twenty-four steers, weighing between 400-450 kg, were divided into four groups with six animals per group. The animals of Group I were implanted once with Torelor (40 mg estradiol + 200 mg trenbolone acetate) and slaughtered at 60 days after implantation. The animals of Group II, III, and IV were implanted twice (60 day interval between implantation) with Torelor and slaughtered 15, 30, and 60 days, respectively, after the second implantation. It should be noted in this study that when Torelor is reimplanted, it is reimplanted in the opposite ear.

The contents of free and conjugated 17β-TBOH and free and conjugated 17α-TBOH were determined in muscle, liver, kidney, fat, and plasma. The determination of the different steroids in the biological matrices was performed by using an HPLC-RIA procedure. (Heister, M., 1986). This was the same analytical method used in the previous study with the same limit of detection.

Tissue residues of free and conjugated 17β- and 17α-TBOH are given in Tables V-VIII:

TABLE V. Free 17β-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

Time of slaughtering after first implantation / after second implantation	60 days	75 days / 15 days	90 days / 30 days	120 days / 60 days
Muscle	188 ± 55	295 ± 88	351 ± 103	282 ± 85
Liver	103 ± 37	219 ± 111	99 ± 47	48
Kidney	256 ± 76	402 ± 96	188 ± 50	163 ± 45
Fat	631 ± 395	1149 ± 473	636 ± 131	826 ± 269

TABLE VI. Conjugated 17β-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

Time of slaughtering after first implantation / after second implantation	60 days	75 days / 15 days	90 days / 30 days	120 days / 60 days
Muscle	35	35	37	18
Liver	551 ± 182	976 ± 330	779 ± 330	330 ± 130
Kidney	82 ± 37	105 ± 22	84 ± 17	63 ± 23
Fat	15	21	12	16

TABLE VII. Free 17α-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

Time of slaughter after first implantation / after second implantation	60 days	75 days / 15 days	90 days / 30 days	120 days / 60 days
Muscle	70 ± 46	61 ± 56	36	48
Liver	141 ± 60	211 ± 108	115 ± 42	47
Kidney	35	43	65 ± 19	48
Fat	20	24	77 ± 16	62 ± 20

TABLE VIII . Conjugated 17α-TBOH Mean Tissue Concentrations in Steers Implanted with TORELOR (ng/kg)

Time of slaughtering after first implantation / after second implantation	60 days	75 days / 15 days	90 days / 30 days	120 days / 60 days
Muscle	63	80 ± 37	88 ± 21	87 ± 21
Liver	1731 ± 475	3085 ± 2183	4652 ± 1513	2055 ± 575
Kidney	183 ± 104	191 ± 90	163 ± 81	95 ± 18
Fat	29	35	76 ± 35	60

Standard deviations given are the absolute standard deviations. Values with no standard deviations are values that are at or below the detection limit of the assay.

The contents of free 17β-TBOH in the muscle, liver, kidney and fat were significantly higher after the bi-sequential implantation when compared to the concentrations after the mono-implantation. Detectable levels of conjugated 17β-TBOH are only found in the liver and kidney.

The contents of free 17α-TBOH are mainly found in the liver. The majority of 17α-TBOH residues are found in the conjugated portion with significant levels primarily in the liver and kidney. (Arts, et al., 1986(b)).

In a third study, eight steers, weighing approximately 280 kg, were simultaneously implanted with Finaplix-S (TBA - 140 mg) and Synovex-S (progesterone - 200 mg + E2β - 20 mg) in the left and right ears, respectively. Four steers were slaughtered 15 days after implantation and the other four steers were slaughtered 30 days after implantation. Samples of fat and muscle were collected for analysis of E2β, progesterone, and 17α-TBOH and 17β-TBOH, and samples of kidney and liver were collected for analysis of 17α-TBOH and 17β-TBOH only.

An RIA procedure was utilized to determine the concentration of 17α-TBOH and 17β-TBOH in muscle, kidney, liver and fat. It should be noted that the unbound residues of trenbolone-17α and trenbolone-17β were measured in the muscle and fat, and the free plus the glucuronide and sulfate conjugates were measured in the liver and kidney.

Tissue residues of 17β-TBOH and 17α-TBOH are given in Tables IX-X:

TABLE IX. 17β-TBOH Mean Tissue Concentrations in Steers Simultaneously Implanted with FINAPLIX and SYNOVEX-S (ng/kg)

	Tissue			
	Muscle	Liver*	Kidney*	Fat
15-Day	147 ± 15	491 ± 39	< 250	421 ± 53
30-Day	241 ± 40	596 ± 108	< 250	505 ± 52

* - The values for the liver and kidney represent the free plus conjugated portion, and the values for the muscle and fat represent only the free portion.

TABLE X. 17α-TBOH Mean Tissue Concentrations in Steers Simultaneously Implanted with FINAPLIX and SYNOVEX-S (ng/kg)

	Tissue			
	Muscle	Liver*	Kidney*	Fat
15-Day	< 15	1128 ± 242	< 250	51 ± 14
30-Day	< 15	1045 ± 165	< 250	< 30

* - The values for the liver and kidney represent the free plus conjugated portion, and the values for the muscle and fat represent only the free portion.

Standard deviations given are the absolute standard deviations. Values with no standard deviations are values that are at or below the detection limit of the assay.

The contents of the free plus conjugated portion of 17β-TBOH in the liver and the free portion in the fat and muscle were significantly higher thirty days after implantation as compared to 15 days after implantation. No detectable residues of 17β-TBOH were found in the kidney.

The only significant residues of the free plus conjugated portion of 17α-TBOH were found in the liver. (Herschler, R.C. 1988).

Heifers

Twenty-four heifers weighing approximately 280 kg were implanted with Finaplix, which contains 300 mg of TBA. Six heifers were slaughtered at each of the following times after implantation: 15, 30, 60, and 75 days. Free and conjugated 17β-TBOH, 17α-TBOH, and e2β were determined in muscle, liver, kidney, fat, and plasma. Conjugated 17α-TBOH and conjugated e2α were determined in urine.

Tissue residues of free and conjugated 17β- and 17α-TBOH are given in Tables XI-XIV:

TABLE XI. Free 17β-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	526 ± 237	528 ± 162	530 ± 310	1091 ± 546
30-Day	645 ± 328	440 ± 148	445 ± 195	1021 ± 535
60-Day	152 ± 24	253 ± 67	340 ± 72	345 ± 164
75-Day	187 ± 103	110 ± 63	145 ± 66	158 ± 109

TABLE XII. Conjugated 17β-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	60	1031 ± 650	179 ± 62	31
30-Day	75	972 ± 470	167 ± 38	46
60-Day	34	909 ± 268	144 ± 34	31
75-Day	97 ± 34	499 ± 176	33	30

TABLE XIII. Free 17α-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	73 ± 78	440 ± 192	144 ± 87	152 ± 48
30-Day	102 ± 106	286 ± 78	155 ± 47	113 ± 54
60-Day	60	63 ± 30	57	93 ± 19
75-Day	42	71 ± 25	26	70 ± 27

TABLE XIV. Conjugated 17α-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

	Tissue			
	Muscle	Liver	Kidney	Fat
15-Day	75	4255 ± 1729	464 ± 353	62
30-Day	59	2920 ± 1130	309 ± 176	60
60-Day	20	1699 ± 755	200 ± 103	40
75-Day	81	1572 ± 733	242 ± 107	44

Standard deviations given are the absolute standard deviations. Values with no standard deviations are values that are at or below the detection limit of the assay.

The concentration of free 17β-TBOH in muscle, liver, and kidney are comparable at 15 days after implantation. The concentration in fat was almost double the other tissues. At 60 days after implantation, the concentration of free 17β-TBOH had significantly decreased as compared to levels at 15 or 30 days after implantation.

Detectable levels of conjugated 17β-TBOH were only found in liver and kidney. Free 17α-TBOH was found in muscle and kidney until 30 days after implantation, and in liver and fat throughout the testing period. (Arts, et al., 1986(c)).

In a second study, thirty heifers weighing approximately 270 kg each were divided into five groups with six animals per group. The heifers in Group I served as a control group

and the animals in Group II-V were implanted twice with Finaplix (300 mg TBA) (60 day interval between implantation). The animals were slaughtered 0, 15, 30, and 60 days, respectively, after the second implantation. It should be noted in this study that when Finaplix is re-administered it is re-implanted in the opposite ear.

The contents of free and conjugated 17β-TBOH, free and conjugated 17α-TBOH and free and conjugated estradiol-17β were determined in muscle, liver, kidney, fat, and plasma. The determination of the different steroids in the biological matrices was performed by using an HPLC-RIA procedure. (Heister, M., 1986).

Tissue residues of free and conjugated 17β- and 17α-TBOH are given in Tables XV-XVIII:

TABLE XV. Free 17β-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

Time of slaughtering after first implantation after second implantation	60 days 0 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	164 ± 143	460 ± 196	210 ± 70	268 ± 116
Liver	95 ± 71	331 ± 150	212 ± 84	181 ± 125
Kidney	176 ± 162	586 ± 221	259 ± 129	156 ± 91
Fat	523 ± 502	2258 ± 980	716 ± 188	511 ± 224

TABLE XVI. Conjugated 17β-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

Time of slughtering after first implantation after second implantation	60 days 0 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	48	25	26	23
Liver	385 ± 378	1172 ± 571	1091 ± 353	1029 ± 480
Kidney	69	137 ± 76	123 ± 23	128 ± 23
Fat	14	8	10	17

TABLE XVII. Free 17α-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

Time of slaughtering after first implantation after second implantation	60 days 0 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	53	96 ± 24	44	45
Liver	97 ± 54	247 ± 134	256 ± 78	187 ± 115
Kidney	37	110 ± 51	72 ± 30	44
Fat	21	60	86 ± 32	77 ± 19

TABLE XVIII. Conjugated 17α-TBOH Mean Tissue Concentrations in Heifers Implanted with FINAPLIX (ng/kg)

Time of slaughtering after first implantation after second implantation	60 days 0 days	75 days 15 days	90 days 30 days	120 days 60 days
Muscle	64	59	78 ± 11	74
Liver	1052 ± 1026	4178 ± 1791	3232 ± 462	2376 ± 968
Kidney	116 ± 78	245 ± 88	339 ± 199	212 ± 71
Fat	14	25	57	57

Standard deviations given are the absolute standard deviations. Values with no standard deviations are values that are at or below the detection limit of the assay.
The highest concentration of free 17β-TBOH was found in the fat and was more than three times the residue level found in muscle, liver and kidney, which all contained comparable levels. Detectable levels of conjugated 17β-TBOH were found in the liver.

The only significant concentrations of free 17α-TBOH and conjugated 17α-TBOH were found in the liver and kidney, with levels of up to 4,000 ng/kg in the liver.

In almost all cases, the concentration of either free or conjugated 17α-TBOH or 17β-TBOH was the highest in the heifers slaughtered 15 days after the re-implantation.

Calves

Twenty-four calves, twelve male and twelve female, were implanted with Revalor (140 mg trenbolone acetate + 20 mg estradiol); three calves of each sex were slaughtered at the following withholding periods: 15, 30, 50 and 70 days. Eight calves were used as controls. Two males and two females were slaughtered at withholding periods of 30 and 70 days. Liver and kidney were analyzed by RIA for free and conjugated 17α-TBOH and 17β-TBOH. Muscle was analyzed for total (free plus conjugate) 17α-TBOH and 17β-TBOH. There was no significant sex-related difference in TBOH levels. The combined results are summarized in Tables XIX and XX.
(Roberts and Cameron, 1986)

TABLE XIX - 17β-TBOH Mean Tissue Concentrations In Calves Implanted with REVALOR (ng/kg)

	Muscle	Liver		Kidney	
	Total*	free	conjugate	free	conjugate
15-Day	237 ± 87.5	414 ± 178	404 ± 198	423 ± 208	240 ± 43.7
30-Day	228 ± 108	908 ± 404	366 ± 112	586 ± 52.7	207 ± 47.6
50-Day	261 ± 91.6	787 ± 413	366 ± 95.7	226 ± 156	198 ± 50.4
70-Day	219 ± 125	763 ± 226	436 ± 56.9	389 ± 211	252 ± 61.5

* Sum of free and conjugate 17β-TBOH.

TABLE XX - 17α-TBOH Mean Tissue Concentrations In Implanted with REVALOR (ng/kg)

	Muscle	Liver		Kidney	
	Total*	free	conjugate	free	conjugate
15-Day	81.2 ± 39.6	982 ± 245	1202 ± 598	322 ± 184	312 ± 283
30-Day	105 ± 43.7	1078 ± 353	754 ± 315	196 ± 90.8	221 ± 34.0
50-Day	66.6 ± 32.5	683 ± 301	584 ± 226	193 ± 54.6	139 ± 37.7
70-Day	44.2 ± 16.5	540 ± 149	733 ± 206	142 ± 37.7	91.6 ± 1.92

* Sum of free and conjugate 17α-TBOH.

METHODS OF RESIDUE ANALYSIS

General

Several analytical techniques have been utilized to quantitate levels of trenbolone in plasma, excreta and tissues. Although economical, TLC procedures are limited to sensitivities of 10-100 ppb. HPLC and GC-MS techniques extend the quantitation levels to 1-10 ppb. RIA techniques further extend the quantitation levels to 0.1-1 ppb and are able to measure both the 17α-TBOH and 17β-TBOH metabolites. All analyses presented in the previous residue studies were conducted using RIA procedures. (Heister, 1986; Hoffman and Ryan, 1978; Hoffman and Oettel, 1976; Jouquey, et al., 1983; O'Keefe, 1984a and 1984b)

Radioimmunoassay RIA

Homogenates of the tissues are extracted with toluene:ether (7:3) in order to separate the free and conjugated steroids. The conjugated steroids are incubated with glucuronidase and sulfatase and the free steroids are extracted with toluene:ether. The steroids are purified by solid phase chromatography, separated by HPLC and quantitated by RIA. The reported detection levels in tissues for free 17β-TBOH, conjugated 17β-TBOH, free 17α-TBOH and conjugated 17α-TBOH are 70, 75, 60, and 75 ppt, respectively. (Arts, et al., 1986(c)).

APPRAISAL

The residue levels in muscle and liver of 17β-TBOH, 17α-TBOH and their conjugates at 30 days after implantation or reimplantation are summarized in Table XXI for steers, heifers and calves. Although the dosage levels and the withholding periods are not the same in the six experiments, the residue data are useful in determining the qualitative nature of the soluble residues in the tissues.

For muscle tissue, the data collected in steers, heifers and calves indicate that almost all of the soluble residue is 17β-TBOH. In the muscle, the ratio of 17β-TBOH to 17α-TBOH ranges from about 10 for the steer to 3 for the heifer to 2 for the calf. For liver tissue, the amount of free 17β-TBOH and free 17α-TBOH in the steers, heifers and calves are comparable; however, when the conjugates are included, the combined 17β-TBOH is 30-60% of the combined 17α-TBOH.

In summary, the primary residue in muscle is 17β-TBOH but the free 17α-TBOH is not an insignificant percent of the residue. In the liver, the primary extractable residue is 17α-TBOH, but the 17β-TBOH makes up a significant portion of the combined extractable residues. Together all free residues total 0.25-0.75 ppb in the muscle and 0.2-2.0 ppb in the liver.

TABLE XXI - Muscle and Liver Concentrations in Steers, Heifers and Calves Thirty Days after Implanted with Trenbolone Acetate (ng/kg)

		Muscle		Liver	
		17β-TBOH	17α-TBOH	17β-TBOH	17α-TBOH
Steer :	free[1]	272	9	323	226
	conjugate[1]	43	10	772	1708
	free[2]	351	36	99	115
	conjugate[2]	37	88	779	465
	free3	241	<15	596*	1045*
	conjugate[3]	----	----	----	----
Heifer:	free[4]	645	102	440	286
	conjugate[4]	75	59	972	2920
	free[5]	210	44	212	256
	conjugate[5]	26	78	1091	3232
Calves:	free[6]	228*	105*	908	1078
	conjugate[6]	-	-	366	754

1 - Steers were implanted with Torelor (200 mg TBA + 40 mg E2β).

2 - Steers were implanted twice with Torelor and the values reflect 30 days after reimplantation.

3 - Steers were simultaneously implanted with Finaplix-S (140 mg TBA) and Synovex-S (200 mg progesterone + 20 mg E2β).

4 - Heifers were implanted with Finaplix (300 mg TBA).

5 - Heifers were implanted twice with Finaplix (300 mg TBA) and the values reflect 30 days after reimplantation.

6 - Calves were implanted with Revalor (140 mg TBA + 20 mg E2β).

* These values represent the sum of free and conjugate 17β-TBOH and 17α-TBOH, respectively.

REFERENCES

Arts, C.J.M., van Baak, M.J., Huisman, I.J., and van Weerden Ir.E.J. (1986a). Residue studies in steers implanted with TORELOR. Unpublished report ILOB-report 561a. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Arts, C.J.M., van Baak, M.J., de Jong, J., and van Weerden Ir.E.J. (1986b). Residue studies in steers after a bi-sequential implantation with TORELOR. Unpublished report ILOB-report 572. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Arts, C.J.M., van Baak, M.J., Huisman, I.J., and van Weerden Ir.E.J. (1986c). Residue studies in heifers implanted with FINAPLIX. Unpublished report ILOB-report 560a. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Arts, C.J.M., van Baak, M.J., de Jong, J., and van Weerden Ir.E.J. (1986d). Residue studies in heifers after bi-sequential implantation with FINAPLIX. Unpublished report ILOB-report 571. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Heister, M. (1986). HPLC-RIA and HPLC-TLC determination of free and conjugated 17β-trenbolone, 17α-trenbolone and estradiol-17β in bile of steers and heifers. Unpublished report, ILOB, Wageningen, Netherlands. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Herschler, R.C., Kushinsky, S., Kearley, E.O., and Henricks, D. (1988). Concentrations of estradiol-17β, progesterone and trenbolone in tissues of feedlot steers following simultaneous implantation of Finaplix and Synovex-S. Study No. IAS 1139-C878, Syntex Research, Palo Alto, California, and Roussel-UCLAF, Paris, France. Submitted to FAO by Roussel-UCLAF, Paris, France.

Jouquey, A., Mouren, M., and Salmon, J. (1983). Analytical methods for trenbolone. In: Anabolics in Animal Production, Office International des Epizooties, Paris, pp. 423-441.

O'Keefe, M. (1984a). Tissue levels of the anabolic agents, trenbolone and zeranol, determined by radioimmunoassays. Proc. of the Symp. on the Analysis of Steroids, Szeged, Hungary. Akademiai Kiado, Budapest (tubls.). Ed. S. Jorog (1985).

O'Keefe, M. (1984b). Trenbolone levels in tissues of trenbolone acetate-implanted Steers: radioimmunoassay determination using different antisera. Br. Vet. J. 140, 592-599.

Roberts, N.L. and Cameron, D.M. (1986). Steroid Levels in tissues of veal calves following implantation with Implix BM/BF and/or Revalor lactose. Unpublished report No. RSL/686 for Huntington Research Centre, Huntington, Cambridgeshire, England. Submitted to FAO by Roussel-UCLAF, Romainville, France.

Windholz, M. ed. (1983). The Merck Index 10th Edition. Rahway, N.J., Merck and Co.

ANNEX I

RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA

Substance	Acceptable Daily Intake (ADI) for human beings	Recommended Maximum Residue Level (MRL)
Anthelminthic drug		
Albendazole	0-0.05 mg/kg of body weight	Muscle, fat and Milk 0.1 mg/kg Liver and kidney: 5 mg/kg
5-Nitroimidazoles		
Dimetridazole	Not allocated[1]	No MRL allocated[2]
Ipronidazole	Not allocated[1]	No MRL allocated[2]
Metronidazole	Not evaluated[3]	No MRL allocated[2]
Ronidazole	0-0.025 mg/kg of body weight[4]	No MRL allocated[5]
Antimicrobial sulfonamides		
Sulfadimidine	0-0.004 mg/kg of body weight[4]	Meat, Liver, kidney, and fat: 0.3 mg/kg as total residue; 0.1 mg/kg as sulfadimidine Milk: 0.05 mg/L as total residue; 0.025 mg/L as sulfadimidine
Sulfathiazole	Not allocated[1]	No MRL allocated[2]
Growth promoter		
Trenbolone acetate	0-0.02 µg/kg of body weight	Muscle: 2 µg/kg as β-trenbolone Liver: 10 µg/kg as α-trenbolone
Trypanocides		
Diminazene	Not allocated[1]	No MRL allocated[2]
Isometamidium	Not allocated[1]	No MRL allocated[2]

1 Insufficient toxicological information was available to establish an ADI.

2 MRLs were not established because: (1) an ADI was not allocated; and (2) insufficient information was available.

3 This substance was not evaluated because toxicological data were not made available to the Committee.

4 Temporary acceptance.

5 Insufficient information was available to establish MRLs.

6 This compound was on the agenda under the name "sulfamethazine", but was evaluated under the name "sulfadimidine", which is the international non proprietary name for it.

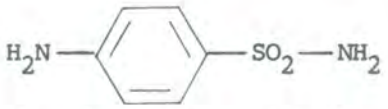
ANNEX II

GENERAL CONSIDERATIONS FOR SULFADIMIDINE AND SULFATHIAZOLE

INTRODUCTION

This Annex was prepared to discuss the many similar properties, uses, metabolism, parmacokinetics and analytical methods for Sulfadimidine and Sulfathiazole. Individual residue monographs for each drug are in this volume.

Both sulfadimidine and sulfathiazole are derivatives of p-aminobenzenesulphonamide



There are at least fifty other derivatives listed in the Merck Index and they are commonly referred to as sulfonamides or sulfas. They all exhibit antibacterial activity and many sulfonamides also are used as coccidiostats in animal production.

The two drugs are broad spectrum antibiotics which are not difficult to synthetize, thus they are not expensive to manufacture and because they are out of patent they are widely available from many manufacturing sources.

An overview would indicate that they are widely used in animal production both as therapeutic and technological drugs. If they were used as generally recommended then the residues should not be a hazard to public health, however it is well knowm that sulfonamides give rise to a large number of drug violations in farm animal products, primarily because the use often differs from that recommended. The metabolism and residue concentrations of sulfadimidine are well documented and a defined time to near zero residues or a set violative level could be established. The information on sulfathiazole is less complete. Although it is possible to set safe levels and withdrawal times based on properly carried out residue studies one should always be mindful that these drugs are often used in such a way that the residues are at higher levels than is acceptable.

Some of the residue data discussed is derived from studies where combined preparations containing either drug were used with other drugs. Most information is only available for the use of the single preparation of each drug and although it might be assumed that the concentration of residues is not very different for single or combined preparations, more caution might be needed in the case of combined preparations where the known effects of the combined preparation usually are greater than the sum of the effects of the single preparations of the constituent drugs.

USE IN FARM ANIMALS

Sulfonamides have a bacteriostatic action. They inhibit bacterial growth by preventing p-aminobenzoic acid from being incorporated into the pteroylglutamic acid (folic acid) molecule. Therapeutic preparations may contain more than one sulfonamide or be combined with other drugs; e.g. a typical preparation as used in the UK for treating enteric conditions in calves and pigs contains in a 30 ml solution: 0.6g sulfathiazole, 0.6g sulfaguanidine, 0.8g phthalysulfathiazole, 3g Kaolin, 200mg streptomycin sulphate and 67mg neomycin sulfate. Trimethoprim is often combined with a sulfonamide to produce a synergic effect which is both bactericidal and has broad spectrum activity.

Therapeutic Uses

Both drugs are highly effective against a wide range of bacterial, protozoal and rickettsial organisms. They are particularly useful in cases of septicaemia, pneumonia, enteritis and diseases of calves caused by Salmonella, Pasteurella and coliform organisms; against necrotyic enteritis in pigs and "foul in the foot" in cattle; coccidiosis in sheep and cattle and treatment - via the drinking water - of caecal and intestinal coccidiosis in poultry and rabbits; also coryza and fowl cholera. The injectable forms have also been used successfully in treating heart-water and tick-borne fever in lambs.

Use as a Technological Drug

Although sulfonamides are widely used to treat enteric conditions their use for preventing enteric diseases and in particular Atrophic Rhinitis in pigs associated with Bordatella bronchiseptica falls into the grey area of whether the compound is being used primarily as a technological drug to improve feed efficiency and liveweight gain or prophylactically as a therapeutic agent.

Sulfonamides including sulfadimidine and sulfathiazole are widely used as in-feed additives for pigs and fed to the pork and bacon pigs throughout most of their life span. The dose rate is usually about 110 g sulfonamide per kg feed. Again the commercial preparations may contain other antibiotics.

PHARMACOKINETICS OF SULFONAMIDES

The sulfonamides are organic acids which are well absorbed from the gastrointestinal tract, except the enteric compounds. They are widely distributed throughout the body and penetrate cell membranes in accordance with their degree of ionisation and lipid solubility. They are eliminated by a combination of renal excretion and biotransformation processes. Sulfonamides bind readily to blood proteins especially albumin. The fate of the sulfonamides in the kidneys involves glomerular filtration of the unbound molecules in the plasma, active carrier-mediated excretion (proximal tubule) of the ionised moiety, and reabsorption, by passive diffusion of the lipid soluble non-ionised fraction. The extent of reabsorption is determined by the pKa and lipid solubility of the sulfonamide and the pH of the tubular fluid. Urinary alkalisation decreases reabsorption by promoting ionisation within tubular fluid, and increases the solubility of the sulfonamide and its acetyl derivatives in the urine. The normal urinary reaction of herbivores is alkaline.

The pathways of biotransformation include acetylation of the aromatic amino group, which takes place in reticuloendothelial cells of the liver and other tissues, and hydroxylation of the aromatic ring which may, in turn, be conjugated with glucuronic acid. Both oxidation and conjugation are catalysed by hepatic microsomal enzymes. The N-glucose-sulfonamide and the desamino derivatives are also formed as metabolites.

Thus the major residues excreted in the urine are the parent drug, the N-acetyl and N-glucose derivatives, the desamino derivatives and water soluble conjugates.

The kinetics of excretion of sulfonamides into urine and the rate of disappearance from plasma follow first order processes. In the simplest models one equation is sufficient but other models use two competing first order equations, one for the elimination of parent drug and a second equation for the acetylation of the parent drug.

The pharmacokinetics of sulfonamides is complicated by the recycling of the drug by contact with or ingestion of contaminated bedding and housing following the excretion of drug by treated animals. This is especially a problem with pigs which are naturally coprophagic and may also happen with poultry. Where large doses are given to humans it is essential to maintain an adequate urinary flow otherwise crystalluria occurs and the drug elimination patterns do not fit the kinetic models.

RESIDUES

Introduction

There are many similarities in the metabolism and residue patterns of the two drugs and many of the analytical methods are common to both.

Because the sulfonamides have been widely used for more than forty years the information on residues is not determined by the modern and more acceptable techniques used today. One major study using C-14 radiolabelled sulfadimidine in pigs was commissioned by the Food and Drug Administration, USA (study No. 224-81-0007) and carried out in 1981. The full report of the study was published in 1984, FDA 224-81-005. This radiometric study of the residues provides the most comprehensive information on sulfonamide residues. Radiometric studies using sulfadimidine in other species or using sulfathiazole in any species have not been reported.

Metabolism

General metabolism of sulfonamides includes the acetylation of the aromatic amino group in the reticuloendothelial cells of the liver and in other tissues yields N-acetyl-derivatives, which are major metabolites of both drugs.

The desamino-sulfonamides have also been identified as residues of the parent drug in cattle (Wooley & Siegel, 1982) and may be the preferred route of metabolism in poultry (Kietzmann, 1981) but do not seem to be an important metabolic product in humans. The desamino-metabolite is thought to be formed by bacteria in the gut following oral administration of the drug. Because it is less polar than the other metabolites the desamino-metabolite is cleared more slowly and this possibly explains why desamino-sulfadimidine is present at a higher percentage of the residues as withdrawal time increases.

Small amounts (<5% of total residues) of polar metabolites are produced but have not been identified in most studies, but it is thought that they are produced by the hydroxylation of the aromatic ring which may be further conjugated with glucuronic acid.

METHODS FOR MEASURING RESIDUES

Classical method using diazotization

The aromatic amino group common to sulfonamides renders them susceptible to diazotization and coupling with specific colour reagents. The method of Bratton & Marshall, (1939), was modified by Annino (1961) and has been widely used to produce much of the data available on the residues of sulfonamides. Unfortunately the method fails to detect the Desamino-derivative because this metabolite has lost the amino group.

The sulfonamide is extracted into a protein-free solution and the drug diazotized with nitrous acid. The excess nitrous acid is destroyed with ammonium sulphamate and the diazonium salt is then coupled with N-(1-naphthyl)ethylenediamine to form a red dye. The colour is measured at 550 nm and interpolated from a standard curve prepared with pure sulfonamide.

The main metabolite of sulfadimidine and sulfatriazole is the acetyl-derivative and because the acetyl group is attached to the amine group it blocks diazotization. To overcome this the protein free extract is hydrolysed with hydrochloric acid which liberates the free amine and then the total residue of sulfonamide plus acetyl-derivative is determined. If the extract is not hydrolysed then the amount of the drug can be determined separately and the amount of residue due to acetyl-derivative can be determined by difference.

Each sulfonamide forms a different product and will not necessarily produce the same amount of colour, thus different standards should be used for specific drugs.

The sensitivity of the method is 0.050 - 0.1 ppm.

Tishler et al. (1968) also published a method based on the Bratton-Marshall reaction. Tissues and milk were extracted into chloroform/acetone and then hydrochloric acid. An aliquot off the acid extract was dried and used to develop the same diazotized products as described by Annino (see above). The method was suitable for all species of tissues and was sensitive to at least 0.1 ppm.

HPLC Methods

High performance liquid chromatography (HPLC) methods all follow the same pattern with only minor variations; - extraction of the drug into an organic phase - a clean up procedure making use of the amphoteric properties of sulfonamides, usually by dissolving or extracting the sulfonamide into strong acid or base and washing with a non-polar solvent-the pH of the extract is adjusted to 5.5-6 and the sulfonamide is extracted into a polar organic solvent. An aliquot is dissolved in a solution suitable for injection onto a C18 column and eluted with aqueous solutions of polar organic compounds (e.g. methanol or acetonitrile) and salts (e.g. acetate or phosphate).

The sensitivity of the methods varies but the most sensitive claim 0.01 ppm. For example:

Source	Drug	Tissue	Lower limit Sensitivity (ppm)
Boisseau (1988)	SMZ	Eggs, Milk	0.01
Weber & Smedley (1988)	SMZ	Milk	0.01
Lutchefeld (1976)	SMZ	Feeds	ca 0.2
Bachman et al (1976)	SMZ	Feeds	ca 0.5

Gas Cromatography (GC)

GC methods have been developed for identifying and measuring residues of sulfonamides and their metabolites. The principles of the method are the same as for HPLC except that a methylation step using diazomethane is necessary to produce the N-methyl derivative which is stable in the GC system. The method is suitable for most sulfonamides and also for the N-acetyl and desamino metabolites. (Matusik et al, (1982); Manuel & Stella, (1981), Holtmannspotter and Thier, (1982).

The lower limit of sensitivity is 0.01-0.05 ppm and is used successfully in the United Kingdom to identify sulfonamide residues.

Thin Layer Chromatography (TLC)

There are a number of TLC methods for measuring residues of sulfonamides. The sulfonamides are extracted using combinations of solvent/solvent partition especially making use of the amphoteric nature of the drugs and small column chromatography (e.g. Sep-Pak columns). The extracts are run on TLC in one or two dimensions and the spots visualised either with fluorescamine or through the Bratton-Marshall diazotization reaction and coupling with N-(1-naphtyl) ethylenediamine. The lower limit of sensitivity of the methods is ca. 0.05 ppm. A TLC method is used in many countries including Holland, Mexico and the USA, for regulating residues of sulphonamides. For TLC methods see: Haagsa et al, (1984); Torda, (1988); Sanchez, (1988); Bevill et al., (1977a); Thomas et al, (1981).

The USA is carrying out extensive tests using a TLC method with fluorescence detection to regulate sulfa residues at slaughterhouses and on the farms. The test is called the "SOS (Sulpha on Site) test" and is claimed to have helped reduce the number of violations seen with sulfa drugs. Urine is analysed by running a sample on a TLC plate and visualising the developed plate under UV-light. The test is calibrated to give a positive when the concentration of sulfonamide in urine is equivalent to a concentration in either liver or muscle <0.1 ppm. The method can be performed in a relatively short time by technicians. The results were shown to agree with those obtained by more laboratory orientated methods.

Enzyme Immunoassay

The introduction of immunoassay methods for the detection of sulfa residues is still under development. Immunoassay tests are continually coming to the market which are specific, rugged, cheap and give rapid answers. Ideally an aspecific immunoassay test is needed which will detect any one of the sulfonamides likely to be found in farm animals. Such a system is still being developed but there are available rapid enzyme immunoassay tests for sulfadimidine. Sulfadimidine can be detected in under 20 minutes in samples of urine, serum or animal feed with lower limits of sensitivity of 0.5 ppm in urine, 0.16 ppm in serum and 1.2 ppm in feed. The tests are quite simple and no specialist technical training is needed to carry them out.

Some government agencies/departments have examined these tests and found them suitable for routine screening purposes and are using them in surveillance schemes.

Mass Spectrometry (MS)

The use of MS for the unambiguous identification and quantitation of drugs is increasing. This desirable situation is well developed for sulfonamides. The UK Ministry of Agriculture has published the results of applying either GC-MS or Tandem-MS (MS-MS) to screening tissues from cattle, pigs and sheep. Fifteen compounds including sulfadimidine and sulfathiazole were known to be detectable by the GC-MS method with the lower limit of sensitivity (LLS) of 0.01 ppm. Five compounds including sulfadimidine were known to be detectable by tandem-MS with an LLS of 0.05 ppm. (UK, MAFF (1987), Food Surveillance Paper, No. 22).

REFERENCES

Annino J.S., (1961), Sulphonamides, in Standard Methods of Clinical Chemistry, Ed by Sligson D.E., New York, Acad. Press, vol. 3, pp 200-206.

Bachman, W.J., Ponder, C., Scott, E. and Thompson, D.W. (1976), Lab. Info. Bull, no 2226, Atlanta Regional Office, Food and Drug Administration (FDA), USA.

Bevill, R.F., Sharma, R.M., Meachum, S.H., Wozniak, S.C., Bourne, D.W.A. and Dittert, L.W. (1977a), Am. J. Vet. Res.38 973-977.

Boisseau, J. private communication (1988).

Bratton, A.C. and Marshall, E.K. (1939), J. Biol. Chem. 128,537.

Haagsma, N., Dielman, B. and Gortemaker, B.G.M., (1984), The Veterinary Quarterly, 6, 8-12.

Holtmannspotter, H. and Thier, H.P., (1982), Deutsche Lebensmittel-Rundshau, 78, 347-350.

Kietzmann, M., (1981), Arch. Gelflugelkd. 45, 233-239.

Lutchefeld, R., (1976), Lab. Information Bull no. 1988, Kansas City Field Office, FDA, USA.

Manuel, A.J. and Stella, W.A. (1981) JAOAC, 64, 794-799.

Matusik, J.E., Barnes, C.J., Newkirk, D.R. and Fuzio, T., (1982) JAOAC 65, 828-834.

Sanchez, J.A. (1988). Dept. de Normalizacion de Productos Alimenticios y Agropecuarios. Mexico.

Thomas, M.H., Soroka, K.E. and Simpson, R.M., (1981), J. Agric. Food Chem., 29, 621-628.

Tishler, F., Sutter, J.L., Bathish, J.N. and Hagman, H.E., (1968) J. Agr. Food Chem., 16, 50-53.

Torda, S.B., (1988). Submitted provisional TLC method by Denver District to Lester Crawford, USA.

Weber, J.D. and Smedley, M.D., (1988), HPLC method submitted by Centre for Vet. Medicine, FDA, USA.

Williams, R.T. (1959), in "Detoxification Mechanisms", John Wiley & Sons, Inc., New York, p. 509.

Wooley, J.L. and Siegel, C.W. (1982), Am. J. Vet. Res. 43, 794-799.

UK, MAFF, Food Surveillance Paper, (1987), No. 22.

FAO TECHNICAL PAPERS

FOOD AND NUTRITION PAPERS:

1. Review of food consumption surveys, 1977
   Vol. 1 — Europe, North America, Oceania, 1977 (E*)
   Vol. 2 — Far East, Near East, Africa, Latin America, 1979 (E*)
2. Report of the joint FAO/WHO/UNEP conference on mycotoxins, 1977 (E* F* S*)
3. Report of the joint FAO/WHO expert consultation on the rôle of 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. Guide to JECFA specifications, 1978 (E* F*)

5 Rev. Guide to JECFA specifications, 1983 (E* F*)

6. The feeding of workers in developing countries, 1978 (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, 1978 (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*)
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* S*)
14. Manuals of food quality control
    1 — The food control laboratory, 1979 (Ar* E*) (Revised edition 1986, E*)
    2 — Additives, contaminants, techniques, 1979 (E* F*)
    3 — Commodities, 1979 (E*)
    4 — Microbiological analysis, 1979 (E* F* S*)
    5 — Food inspection, 1981 (Ar* E*) (Revised edition 1984, E*)
    6 — Food for export, 1979 (E*)
    7 — Food analysis: general techniques, additives, contaminants and composition, 1986 (E*)
    8 — Food analysis: quality, adulteration and test of identity, 1986 (E*)
    9 — Introduction to food sampling, 1988 (E*)
15. Carbohydrates in human nutrition, 1980 (E* F* S*)
16. Analysis of food and nutrition 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, 1981 (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* 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, 1983 (E* F*)
29. Post-harvest losses in quality of foodgrains, 1983 (E* F)
30. FAO/WHO food additives data system, 1984 (E*)

30 Rev. 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. Specifications for identity and purity of certain food additives, 1986 (E* F***)
35. Review of food consumption surveys, 1985 (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*)
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*)
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, 1989 (E*)
45. Exposure of infants and children to lead, 1989 (E*)
46. Street foods, 1989 (E/F/S*)
47/1. Utilization of tropical foods: cereals, 1989 (E*)
47/2. Utilization of tropical foods: roots and tubers (E***)
47/3. Utilization of tropical foods: trees (E***)
47/4. Utilization of tropical foods: tropical beans (E***)
47/5. Utilization of tropical foods: tropical oilseeds (E***)
47/6. Utilization of tropical foods: sugars, spices and stimulants (E***)

Availability: February 1990

Ar — Arabic
C — Chinese
E — English
F — French
S — Spanish

* Available
** Out of print
*** In preparation

The FAO Technical Papers can be purchased locally through FAO Sales Agents or directly from Distribution and Sales Section, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy.

M-84/T0292E/1/3.90/3000
ISBN 92-5-102933-4