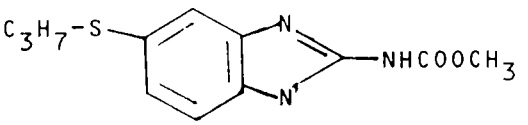
ALBENDAZOLE

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

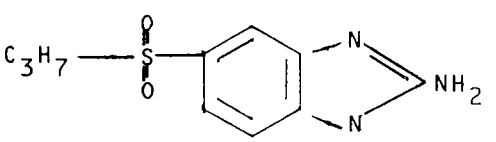
Other properties: White to buff, odourless and stable at room temperature for up to 2 years

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.

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