UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

NOV 30 2000

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of a Metabolism/Pharmacokinetics Study
EPA Reg. No:0F06172
1,2-Benziothiazolin-3-one
Case: 293131
Case Type: Tolerance Petition

From: S. L. Malish, Ph.D., Toxicologist S.L. Malish 11/28/00
Risk Assessment and Science Support Branch (RASSB),
Antimicrobials Division (AD) (7510C)

To: Robert Forrest, PM 5
PM Team Reviewer, Kathryn Boyle
Regulatory Management Branch
Antimicrobial Division

Thru Winston Dang, Team Leader,
Team One, RASSB/AD [7510C]

and

N. Cook 11.30.00

Norman Cook, Chief, RASSB/AD [7510C]

Applicant: Avecia, Inc. Wilmington, DE

Laboratory: Imperial Chemical Industries Ltd, Alderley Park, UK

FORMULATION:

Active Ingredient:	% by Weight
1,2-Benzisothiazolin-3-one	NA

RECOMMENDATIONS:

Phase 1 of the metabolism study is **ACCEPTABLE** and satisfies the guideline requirement for a tier 1 (§ 85-1) General Metabolism study in rats.

Phase 2 of the General Metabolism study [MRID 45124902] is considered to be **UNACCEPTABLE, but ungradable** and does not satisfy the guideline requirement for a tier 1 General Metabolism study OPPTS 870.7485 (§ 85-1).

The executive summaries are listed below. Complete data evaluation documents are attached.

EXECUTIVE SUMMARIES:

1. **[§ 85-1] Metabolism/Pharmacokinetics Study (MRID 45124901)**

In a metabolism study (MRID 45124901) 1,2-Benzisothiazolin-3-one [purity not given], labeled with sulfur 35 ([^{35}S]BIT) was orally administered (assumed to be by gavage) to four male Alderley Park derived Wistar rats in a single dose at 20 mg/kg (Group 1) or to eight male rats of the same strain in a single dose or 2, 3, or 4 repeated daily doses at 10 mg/kg (Group 2). The second phase of the study (MRID 451249-02) concerning the characterization of the metabolites is included as a separate report.

The results reported were based on the analysis of urine and fecal samples collected from the rats in Group 1 and abdominal fat and liver samples from the rats in Group 2. The estimated percentage of radioactivity in the excreta was reported for individual animals every 24 hours for 3 days (feces) or 5 days (urine). The authors report that in Group 1, 91% of the radioactivity was excreted in the urine within 5 days post exposure and 5% was excreted in the feces. In the Group 2 rats, accumulation was not reported in the abdominal fat and ≤0.22 ppm BIT was recovered from the liver tissue samples.

Phase 1 of the metabolism study is **ACCEPTABLE** and satisfies the guideline requirement for a tier 1 (§ 85-1) Metabolism and Pharmacokinetics study in rats. Phase 1 of the study must be read in conjunction with phase 2 [metabolites characterization] of the Metabolism and Pharmacokinetics study [MRID 45124902].

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2. **[§ 85-1] Metabolism/Pharmacokinetics Study (MRID 45124902)**

In a metabolism characterization study (MRID 45124902), 1,2-benzisothiazolin-3-one (BIT) [purity not given], labeled with sulphur-35 ([^{35}S]BIT) was administered to groups of rats and a single dog. A group of ten male Alderley Park derived Wistar rats received a single oral dose of 400 mg/kg (0.23 µCi). Two male rats of the same strain received an intraperitoneal dose of 2.2 µCi, and in an addition, single Beagle dog [sex not specified] was given a gelatin capsule containing 1.2 mg/kg (4.6 µCi). The first phase of the study (MRID 45124901) concerning the toxicokinetics is included as a separate report.

The major urinary metabolites were identified from pooled 24 hour urine samples in the rat and dog using thin layer chromatography/ mass spectrometry. Three metabolites were present in the urine samples. Of the three, 2 were positively identified as o-(methylsulphinyl)benzamide [Metabolite 2, 66 to 81%] and o-(methylsulphonyl) benzamide [Metabolite 3, 9 to 19%]. The author concluded that the metabolites present in the urine of both species resulted from the reduction of the nitrogen-sulfur bond, followed by the methylation and oxidation of the sulphur atom. The proposed metabolic pathway for BIT was:

BIT → o-mercaptobenzmide → o-(methylthio)benzamide → o-(methylsulphinyl)benzamide → o-(methylsulphonyl)benzamide

The breakdown of BIT is rapid and is carried virtually to completion in both the rat and dog; qualitatively the metabolites in both species are similar.

Phase 2 of the metabolism study [MRID 45124902] is considered to be **UNACCEPTABLE, but upgradable** and does not satisfies the guideline requirement for a tier 1 Metabolism and Pharmacokinetics study [OPPTS 870.7485 (§ 85-1)]. The study can be upgraded by supplying the characterization of the unidentified metabolite, the dose level used in the intraperitoneal study and the sex of the dog. Phase 2 of the study must be read in conjunction with phase 1 of the Metabolism and Pharmacokinetics study [MRID 45124901].

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EPA Reviewer: Steven L. Malish, Ph.D., Toxicologist
Team 1 RASSB/Antimicrobials Division (7510C)
Secondary Reviewer: Jonathan Chen, Team 3
RASSB/Antimicrobials Division (7510C)

S.L. Malish 11/28/00
Jonathan Chen 11/28/00

DATA EVALUATION RECORD

STUDY TYPE: Metabolism and Pharmacokinetics/Rat
[§ 85-1, OPPTS 870.7485]

DP BARCODE: D267647
P.C. CODE: 098901

SUBMISSION CODE: S582879
CASE TYPE: Tolerance Petition

TEST MATERIAL (PURITY): 1, 2-Benzisothiazolin-3-one (purity not provided), BIT

SYNONYMS: Proxel, Proxel XL, 1, 2-Benzisothiazolin-3 (2H)-one, IPX, Proxan

CITATION: Conning, D.M. (1972). 1,2-Benzisothiazolin-3-one: Excretion and Tissue Retention in the Rat. Imperial Chemical Industries Limited, Industrial Hygiene Research Laboratories, Alderley Park, UK. HO/IH/P/3. MRID 45124901. Unpublished.

SPONSOR: Avecia, Inc. (Wilmington, DE)

EXECUTIVE SUMMARY:

In a metabolism study (MRID 45124901) 1,2-Benzisothiazolin-3-one [purity not given], labeled with sulfur 35 ([^{35}S]BIT) was orally administered (assumed to be by gavage) to four male Alderley Park derived Wistar rats in a single dose at 20 mg/kg (Group 1) or to eight male rats of the same strain in a single dose or 2, 3, or 4 repeated daily doses at 10 mg/kg (Group 2). The second phase of the study (MRID 451249-02) concerning the characterization of the metabolites is included as a separate report.

The results reported were based on the analysis of urine and fecal samples collected from the rats in Group 1 and abdominal fat and liver samples from the rats in Group 2. The estimated percentage of radioactivity in the excreta was reported for individual animals every 24 hours for 3 days (feces) or 5 days (urine). The authors report that in Group 1, 91% of the radioactivity was excreted in the urine within 5 days post exposure and 5% was excreted in the feces. In the Group 2 rats, accumulation was not reported in the abdominal fat and ≤0.22 ppm BIT was recovered from the liver tissue samples.

Phase 1 of the metabolism study is **ACCEPTABLE** and satisfies the guideline requirement for a tier 1 (§ 85-1) Metabolism and Pharmacokinetics study in rats. Phase 1 of the study must be read in conjunction with phase 2 [metabolites characterization] of the Metabolism and Pharmacokinetics study [MRID 45124902].

COMPLIANCE: This study included a signed and dated "Statement of No Data Confidentiality Claim" and a signed and dated GLP statement. Quality Assurance and Flagging statements were not provided.

I. MATERIALS AND METHODS

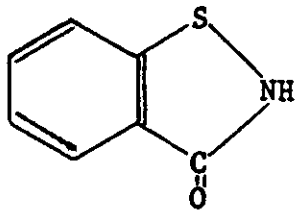
A. MATERIALS:

1. Test Compound:

Radioactive Compound: 1, 2-Benzisothiazolin-3-one, labeled with sulphur-35 ([^{35}S]BIT)
Radiochemical purity: Single radioactive component was detected with TLC

Specific activity: 1.3 mCi/mmole
Lot/Batch: Not reported

Non radioactive Compound: 1, 2-Benzisothiazolin-3-one
Purity: Not available
Lot/Batch No.: Not reported
Description: Active component of Proxel CRL (an industrial biocide)
Contaminants: Not assessed



1,2-Benzisothiazolin-3-one

2. Vehicle: Radiolabelled test compound was dissolved in aqueous ethanol.

3. Test animals:
Species: Rat
Strain: Alderley Park derived Wistar specific pathogen free
Age and weight at study initiation: Not reported
Source: Not reported
Housing: Individual metabolism cages
Diet: Not reported
Water: Not reported
Environmental

B. STUDY DESIGN AND METHODS:

1. Group Arrangements

Animals were assigned to the test groups presented in Table 1.

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TABLE 1. Dosing groups for pharmacokinetic studies for [^{35}S]BIT

Test Group	Dose of labeled material	Number/sex	Remarks
High single dose (Group 1)	20 mg/kg	4/Male	Excreta analyzed daily, Urine was collected up to 120 hours, feces were collected up to 72 hours.
Low repeated dose (Group 2)	10 mg/kg/day for 1, 2, 3, or 4 days	8/Male (2 per repeat dosing subgroup)	2 animals sacrificed per day, remaining animals were redosed and the procedure was repeated.

2. Dosing and sample collection:

Two separate groups of animals were used in this study. In Group 1, four male Alderley Park derived Wistar rats were orally dosed with 20 mg/kg (0.81 µCi) [^{35}S]BIT and transferred to individual metabolism cages.

In Group 2 , eight male Alderley Park derived Wistar rats were orally dosed (method not specified) with 10 mg/kg (0.76 µCi) [^{35}]BIT. Two animals were killed 24 hours post-exposure and the remaining animals were re-dosed and the procedure repeated until all animals had been sacrificed. Following each sacrifice, portions of the liver and abdominal fat were removed for radiochemical analysis.

a. Toxicokinetic studies

In Group 1, urine and feces were collected separately and excreta were analyzed daily. In Group 2, two animals were sacrificed each day and portions of the liver and abdominal fat were removed for radiochemical analysis.

Alliquots of urine (1 ml) collected from animals in Group 1 were added to a 15 ml scintillator solution of naphthalene, diphenyloxazole, bis-diphenyloxazole, and colloidal silica in p-dioxan.

Fecal samples from Group 1 and liver samples from Group 2 were homogenized in acetone and centrifuged separately. The supernatant was collected and the residue was air dried. The sample was them combusted in oxygen and the liberated [^{35}S]SO_2 absorbed in 2N·NaOH. Aliquots of this solutions were then processed in the same manner as the urine samples.

Three to five gram samples of abdominal fat were homogenized in p-dioxan (4 ml solvent per gram of tissue). The homogenate was then centrifuged and the supernatant was processed in the same manner as the fecal and liver samples.

Radioactivity was measured in the samples using a Packard Tricarb 3002 Liquid Scintillation Spectrometer. A solution of the labeled test compound was used to determine the counting efficiency; therefore, the authors report that there was no need for making a correction for the radiochemical decay of the isotope.

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II. RESULTS

A. Toxicokinetic Studies:

1. Absorption

Based on the recovery of the label in the urine, the test article was well absorbed into the systemic circulation. Approximately 91% of the administered radioactivity was recovered in the urine over a 5-day period following a single oral dose of 20 mg/kg BIT (0.81 μCi), with the majority of the label (75 - 92%) recovered within 24 hours post-administration.

2. Tissue distribution

Tissue distribution data were not reported for Group 1 rats that received a single dose of BIT. In rats that received 10 mg/kg BIT, based on measurements of radioactivity, there was no accumulation of BIT or its sulfur-containing metabolites in the abdominal fat, and BIT levels in the liver ranged from not detected to 0.22 ppm. Individual results of analyses of the fat and liver samples are presented in Table 2. These results show no evidence of accumulation following repeated doses of BIT.

TABLE 2. Distribution of radioactivity in rat abdominal fat and liver after administration of multiple doses of 10 mg ([^{35}S]BIT)\kg[a].

Number of doses	Counts per 20 minutes per 0.5 g of abdominal fat		Radioactive equivalent dose (ppm of administered dose) in liver samples	
0	Sample 1	Sample 2	Sample 1	Sample 2
	603	634	0	0
1	590	611	0	0.02
2	596	608	0.05	0.22
3	726	600	0.12	0.02
4	618	741	0.01	0.04

a Data extracted from Table 2 and Table 3 of the study.

3. Excretion

The authors report that 5 days following a single oral dose of BIT, the mean excretion of radioactivity in the urine was 91% and in the feces was 5%. Radioactivity recovery from urine samples in the four animals was 90.9%, 95.7%, 95.9%, and 81.9%, respectively (Table 3). The highest amounts of excreted material in the urine were seen 24 hours after dosing. Analysis of fecal samples showed the highest amount of radioacitivity was recovered 24 hours after dosing. The cumulative total amount of labeled test material collected in the feces of the four animals by 72 hours post dosing was 0.2%, 2.5%, 2.8%, and 15.2% of the administered dose, respectively, which the authors noted was evidence that BIT is absorbed at variable rates from the

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gastrointestinal tract. Excretion data for the repeated low-dose study were not reported.

TABLE 3. Recovery of radioactivity in excreta of rats after administration of 20 mg([^{35}S]BIT)\kg[a]

Animal	Percentage of dose excreted in urine						Percentage of dose excreted in feces			
	0-24 hr	24-48 hr	48-72 hr	72-96 hr	96-120 hr	**Total**	0-24 hr	24-48 hr	48-72 hr	Total
1	75.4	12.9	1.3	0.8	0.5	**90.9**	0.0	0.2	0.0	0.2
2	87.2	7.1	0.8	0.4	0.2	**95.7**	1.2	1.0	0.3	2.5
3	91.9	3.4	0.4	0.1	<0.1	**95.9**	2.4	0.3	0.1	2.8
4	76.5	4.6	0.5	0.2	<0.1	**81.9**	7.5	7.5	0.2	15.2

a Data extracted from study Table 1 of the study report.

III. DISCUSSION

A. Summary

Two separate groups of rats were administered ([^{35}S]BIT). In Group 1, four male rats were dosed orally with 20 mg/kg of the labeled test substance and samples of the urine and feces were analyzed at 24 hour intervals for 3 days (feces) and 5 days (urine) following exposure. In Group 2, eight male rats were administered 10 mg/kg of the test substance orally. Two animals were sacrificed 24 hours post-administration and portions of the abdominal fat and liver were removed for analysis. The surviving animals were re-dosed and the procedure was repeated.

Radioactivity in the collected samples of tissue and excreta was analyzed using liquid scintillation spectrometer and counting efficiency was determined using a solution of ([^{35}S]BIT) as the internal standard.

For Group 1, the average amount of labeled test material excreted in the urine at five days post-exposure was 91% and the average amount in the feces was 5%. The authors noted that there was evidence that BIT was absorbed at a variable rate from the gastrointestinal tract and that no BIT or its metabolites accumulated in the liver or the abdominal fat. No significant accumulation occurred in the abdominal fat or liver of the animals in Group 2. Since it was determined that ≈96% of the administered dose was found in the excreta, and virtually none in the liver and fat, the collection of the tissues (liver, fat, blood, gastrointestinal tract , kidney, spleen, residual carcass) need not be done.

In the preliminary whole body radioautoraphic study in mice [MRID 4512402], although a different species was used, the study indicated that BIT is rapidly excreted. Five (5) hours after the dose was administered i.v. radioactivity was only detected in the bile, stomach and intestine. Twenty four (24) hours after dosing, the radiolabel excretion of the radiolabel was almost complete, the only activity seen was in the contents in the anterior part of the stomach. This study would support the assumption that no significant accumulation of

radioactivity occurred in the abdominal fat or liver of the animals in Group 2 and that the excretion of

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radioactivity is rapid in the rat.

The amount of radioactivity in expired air was not measured. Given that ≈96% of the radioactivity was present in the urine and feces, the respiratory pathway is probably minor.

B. Study Deficiencies

Major study deficiencies:

- Information regarding the purity of the test article and the homogeneity of the test solutions was not provided.

Minor study deficiencies:

- The age, body weight, and health status of the test animals was not provided.

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EPA Reviewer: Steven L. Malish, Ph.D., Toxicologist
Team 1 RASSB/Antimicrobials Division (7510C)
Secondary Reviewer: Jonathan Chen, Team 3
RASSB/Antimicrobials Division (7510C)

S.L. Malish 11/28/00
Jonathan Chen 11/28/00

DATA EVALUATION RECORD

STUDY TYPE: Metabolism and Pharmacokinetics/Rat and Dog
[§ 85-1, OPPTS 870.7485]

DP BARCODE: D267647
P.C. CODE: 098901
SUBMISSION CODE: S582879
CASE TYPE: Tolerance Petition

TEST MATERIAL (PURITY): 1, 2-Benzisothiazolin-3-one (purity not reported), BIT

SYNONYMS: Proxel, Proxel XL, 1, 2-Benzisothiazolin-3 (2H)-one, IPX, Proxan

CITATION: Dixon, A.M. (1976). 1,2-Benzisothiazolin-3-one: Metabolism in the Rat and Dog. Imperial Chemical Industries, Central Toxicology Laboratory, Alderley Park, UK. CTL/P/227. MRID 45124902. Unpublished.

SPONSOR: Avecia, Inc., Wilmington, DE

EXECUTIVE SUMMARY:

In a metabolism characterization study (MRID 45124902), 1,2-benzisothiazolin-3-one (BIT) [purity not given], labeled with sulphur-35 ([^{35}S]BIT) was administered to groups of rats and a single dog. A group of ten male Alderley Park derived Wistar rats received a single oral dose of 400 mg/kg (0.23 μCi). Two male rats of the same strain received an intraperitoneal dose of 2.2 μCi, and in an addition, single Beagle dog [sex not specified] was given a gelatin capsule containing 1.2 mg/kg (4.6 μCi). The first phase of the study (MRID 45124901) concerning the toxicokinetics is included as a separate report.

The major urinary metabolites were identified from pooled 24 hour urine samples in the rat and dog using thin layer chromatography/mass spectrometry. Three metabolites were present in the urine samples. Of the three, 2 were positively identified as o-(methyl-sulphinyl)benzamide [Metabolite 2, 66 to 81%] and o-(methylsulphonyl) benzamide [Metabolite 3, 9 to 19%]. The author concluded that the metabolites present in the urine of both species resulted from the reduction of the nitrogen-sulfur bond, followed by the methylation and oxidation of the sulphur atom. The proposed metabolic pathway for BIT was:

BIT → o-mercaptobenzmide → o-(methylthio)benzamide → o-(methylsulphinyl)benzamide → o-(methylsulphonyl)benzamide

The breakdown of BIT is rapid and is carried virtually to completion in both the rat and dog; qualitatively the metabolites in both species are similar.

Phase 2 of the metabolism study [MRID 45124902] is considered to be **UNACCEPTABLE, but upgradable** and does not satisfies the guideline requirement for a tier 1 Metabolism and

Pharmacokinetics study [OPPTS 870.7485 (§ 85-1)]. The study can be upgraded by supplying the characterization of the unidentified metabolite, the dose level used in the intraperitoneal study and the sex of the dog. Phase 2 of the study must be read in conjunction with phase 1 of the Metabolism and Pharmacokinetics study [MRID 45124901].

COMPLIANCE: This study included a signed and dated "Statement of No Data Confidentiality Claim" and a signed and dated GLP statement that indicated that the study was completed and reported prior to October 16, 1989 and that GLP requirements were not applicable. Quality Assurance and Flagging statements were not provided.

I. MATERIALS AND METHODS

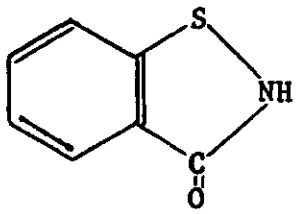
A. MATERIALS:

1. Test Compound:

Radioactive Compound: 1, 2-Benzisothiazolin-3-one, labeled with sulphur-35 ([^{35}S]BIT)
Radiochemical purity: Single radioactive component was detected with TLC
Specific activity: 1.3 mCi/mmole

Lot/Batch: Not reported

Non-radioactive Compound: 1, 2-Benzisothiazolin-3-one



1,2-Benzisothiazolin-3-one

Purity: Not reported
Description: Active component of Proxel CRL (an industrial biocide)
Contaminants: Not assessed
CAS No.: 2634-33-5

2. Vehicle: Corn oil for oral dose in dogs. No information given for oral dose in rats.

3. Test animals:
(a) Species: Rat
Strain: Alderley Park derived Wistar
Age and weight at study initiation: Oral rat study = 200 grams. No additional information reported
Source: Not reported

Housing: Housed collectively for oral exposure. Individual metabolism cages for intraperitoneal exposure.
Diet: Not reported
Water: Not reported
Environmental conditions:
Temperature, humidity, air changes, photoperiod, and acclimation period not reported.

(b) Species: Dog (Beagle)
Strain: Inbred strain
Sex: Not available
Age and weight at study initiation: Age not available. Weight - 15.5 kg
Source: Not available
Housing: Individual metabolism cage
Diet: Twice daily
Water: Not reported
Environmental conditions:
Temperature, humidity, air changes, photoperiod, and acclimation period not reported.

4. Preparation of dosing solutions: The author reported that, 1,2-benzisothiazolin-3-one, labeled with sulphur-35 ([^{35}S]BIT) was obtained from Imperial Chemical Industries Limited, Petrochemicals Division. No other information concerning preparation of the dosing solution was reported. The solution that was used for dosing was also used as an internal standard for determining radiochemical counting efficiency. The radioactivity of the samples was determined using a Packard Tricarb 3002 Liquid Scintillation Spectrometer.

B. STUDY DESIGN AND METHODS:

1. Group Arrangements

Animals were assigned to the test groups presented in Table 1.

TABLE 1: Dosing groups for pharmacokinetic studies for [^{35}S]BIT

Test Group	Dose of labeled material	Number/sex	Remarks
Rats (Oral)	400 mg/kg (0.23 µCi)	10	Housed collectively/24-hour urine sample collected.
Rats (Intraperitoneal)	2.2 µCi	2	Transferred to individual metabolism cages for collection of urine. 24 hour urine sample collected.
Dog (Oral)	1.2 mg/kg (4.6 µCi)	1	Housed in an individual metabolism cage. 24 hour urine sample collected.

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2. Dosing and sample collection:

Oral Study in Rats Ten male Alderley Park derived Wistar rats were orally dosed with 400 mg/kg (0.23 μCi) [^{35}S]BIT. The animals were collectively housed after exposure and a 24-hour collective urine sample was obtained.

Intraperitoneal Study in Rats Two male Alderley Park derived Wistar rats were given intraperitoneal injections of 2.2 μCi [^{35}S]BIT and housed in individual metabolism cages for collection of urine.

Oral Study in a Dog One Beagle dog [sex not specified] was given a gelatin capsule containing 1.2 mg test substance/kg body weight (4.6 μCi) and housed in a metabolism cage.

3. Toxicokinetic Study

Preliminary experiment

A preliminary whole-body autoradiography study of [^{35}S]BIT (specific activity of 25 μCi/ml) was performed in mice. The mice were treated with 0.15 ml (3.75 μCi) i.v. and sacrificed at 5 minutes, 10 minutes, 1 hour, and 24 hours after dosing. Animal tissue was held in contact with X-ray film for 5 weeks

4. Measurement of Radioactivity

No information was provided concerning the methods employed for urine collection (e.g. cage washes, etc.). Radioactivity in urine samples was analyzed using a Packard Tricarb 3002 Liquid Scintillation Spectrometer.

5. Metabolite characterization studies

Fractionation and purification

Urine samples were pooled, freeze-dried and triturated with ethyl acetate. The solution was then filtered, evaporated under reduced pressure at 40°C and the residue was dissolved in water. The pH was adjusted to 9 with saturated sodium bicarbonate and extracted with ethyl acetate. The solvent was then evaporated and the residue dissolved in methanol. Solutions were applied to thin layer plates of silica gel CF and one- or two-dimensionally developed using on of the following solvents:

(1) n-Butanol:ethanol:water
(2) n-Butanol saturated with water
(3) n-Butanol:glacial acetic acid: water
(4) N-Butanol saturated with 2N ammonia
(5) Chloroform:methanol
(6) Methanol:ethyl acetate
(7) Benzene:Methanol
(8) Isopropanol:ammonia::water

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Metabolites were detected under ultraviolet light and radioactive areas were identified with a radiochromatogram scan or radioautograph with Kodak 'Kodirex" X-ray film. Relative distribution of the metabolites was assessed by removing and counting areas of support corresponding to radioactivity.

Metabolites were further purified by chromatography on plates of silica gel HR (500 μ) then characterized by co-chromatography with the test substance and analyzed by mass spectrometry.

Enzymatic hydrolysis of ^{35}S metabolites

Solutions of metabolites were dried, redissolved in 0.2 M sodium acetate buffer, and incubated for 18 hours at 37°C with β-glucuronidase containing aryl sulphatase. The mixture was then evaporated, triturated with methanol, and compared by chromatography with those from a control incubation without enzyme.

Determination of ^{35}sulphate in rat urine

Twenty-four-hour urine samples from the exposed rats were pooled and diluted to 50 ml in water. Sodium sulphate (1 gram), hydrochloric acid, and 0.5 M barium chloride was added and the precipitate was collected. The precipitate was washed with water and acetone and dried in a vacuum desiccator before 100 mg samples were analyzed for radioactivity.

II. RESULTS

A. Toxicokinetic Studies:

In the preliminary radioautography study in the mouse, 5 minutes post-dosing, showed there were high levels of radioacitivity in the blood, lung, brain, and pituitary. At 10 minutes post dosing, high levels of radioactivity were reported in the urine with lower levels in the blood, lung, liver, muscle, kidney, stomach, and intestine. At 1 hour post dosing, levels of radioactivity were reduced in the tissues; however urine levels were still high. Radioactivity was only detected in the bile, stomach, intestines, and urine at 5 hours after dosing. At 24 hours post dosing, excretion was nearing completion with some radioactivity reported in the stomach.

B. Metabolite characterization studies:

No BIT was found in either the rat or dog urine samples.

The results of this study indicate that three metabolites were present in solvent systems 4 and 5 of the methanol extract of urinary metabolites. The identity of Metabolite 1 was not determined, but the author reports that the metabolite did not co-chromatographed with o-(methythio) benzamide. Using mass spectrometry, Metabolite 2 showed a parent ion at m/e 183.0355 corresponding to $C_8H_9NO_2S$, with a peak also occurring at 168.0118 indicating that Metabolite 2 was o-(methyl sulphinyl)benzamide. Metabolite 3 gave the parent ion at m/e 199.0301 corresponding to $C_8H_9SO_3N$. These results suggest that Metabolite 3 was o-

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(methylsuphonyl)benzamide. These results were confirmed by co-chromatography with the authentic compounds in solvent systems 4, 5, 7, and 8.

β-glucuronidase/aryl sulphatase did produce hydrolysis as indicated by chromatography in solvent systems 1 through 8.

A negligible amount of sulphate ion was present in the rat urine when analyzed for radioactivity.

TABLE 2. Metabolite profile in excreta of rats and dogs dosed with ([^{35}S]BIT)[a].

Dose	Metabolites expressed as percent radioactivity in Day 1 Urine		
Compound	Rat (Oral)	Rat (intraperitoneal)	Dog (Oral)
Parent	0	0	0
Identified Metabolite 2: o-(methyl sulphinyl)benzamide.	66	81	68
Identified Metabolite 3: o-(methylsuphonyl)benzamide	9	12	19
Total identified	75	93	87
Unidentified Metabolite 1	25	7	12
Unidentified at origin or at some band	0	0	0
Total unidentified.	25	7	12
Total accounted for [b]	100	100	99
Lost/unaccounted for [c]	0	0	1
Total	100	100	100

a Data extracted from Table 1 of study.
b Total accounted for = (Total identified) + (Total unidentified).
c 100 - (Total accounted for).

III. DISCUSSION

A. Summary

In this metabolism study, 1,2-benzisothiazolin-3-one, labeled with sulphur-35 ([^{35}S]BIT), was administered to groups of male rats and to a single dog [sex not stated]. A group of ten male Alderley Park derived Wistar rats received a single oral dose of 400 mg/kg (0.23 μCi), two male rats of the same strain received an intraperitoneal dose of 2.2 μCi, and one Beagle dog was given a gelatin capsule containing 1.2 mg/kg (4.6 μCi).

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The major urinary metabolites were identified from pooled urine samples using thin layer chromatography. Three metabolites were present in the urine samples. Of the three, two were positively identified as o-(methyl-sulphinyl)benzamide (metabolite 2, 9 to 19% in the rat and dog) and o-(methyl sulphonyl)benzamide (metabolite 3). Metabolite 2 was the predominate metabolite in the rat and dog urine with 66% to 81% of the recovered radioactivity associated with the metabolite. The author concluded that the metabolites present in the urine of both species resulted from the reduction of the nitrogen-sulfur bond, followed by the methylation and oxidation of the sulphur atom. The proposed metabolic breakdown for BIT was :

BIT → o-mercaptobenzmide → o-(methylthio)benzamide → o-(methylsulphinyl)benzamide [Metabolite 2] → o-(methylsulphonyl)benzamide [Metabolite 3]

The radioautography study in the mouse, although suggestive of what may occur in rats, might not be valid for the extent of distribution of BIT.

B. Study deficiencies

Major study deficiencies included the following:

- Metabolite 1 was not identified. This metabolite must be identified or an explanation as to why it was not identified, which is acceptable to the Agency, must be provided.
- In the i.p. study in the rat, the dose level in mg/kg/ must be specified.
- The sex of the dog must be provided.

Minor study deficiencies included the following:

- The age and health status of the test animals was not provided.
- The materials and methods of the preliminary radioautography study in mice were not specified.
- Animals were transferred to individual metabolism cages following exposure; however, there was no mention of rinsing the cages with the appropriate solvent following each collection period to ensure maximum recovery of radiolabel.
- Information regarding the purity of the test article and the homogeneity of the test solutions was not provided.

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