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WASHINGTON, D.C. 20460

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361
AUG 22 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Acute Oral LD50, Acute Dermal LD50, Primary Dermal Irritation, and Dermal Sensitization Studies on 0.25% Brodifacoum Formulation Concentrate; Metabolism Study on Brodifacoum

FROM: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus 8/16/96
Toxicology Branch 2
HED (7509C)

TO: John Redden,
HED (7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 8/16/96
Section Head, Review Section II
Toxicology Branch 2
HED (7509C)

and

Yiannakis Ioannou, Ph.D., Acting Branch Chief
Toxicology Branch 2
HED (7509C)

DP Barcode: D227302 Submission: S506631

Chemical: 112701 Brodifacoum

Action Requested: Review of acute oral LD50 (MRID 44021701), acute dermal LD50 (MRID 44021702), primary dermal irritation (MRID 44021703), and dermal sensitization (MRID 44021704) studies on 0.25% Brodifacoum Formulation Concentrate; and review of a metabolism study on Brodifacoum (MRID 44021705).

EXECUTIVE SUMMARIES:

1. In an acute oral toxicity study (MRID No. 44021701), groups of fasted, young Alpk:APfSD (Wistar-derived) rats, 5/sex were given a single oral dose of Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%) in deionized water at doses of 50, 200, or 500 mg/kg (males), and doses of 100, 150 or 200

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mg/kg (females), and were subsequently observed for 14 days.

LD_{50} Males = 163 (95% C.I.: 97-275) mg/kg
Females = 152 (95% C.I.: 132-175) mg/kg
Combined = not reported

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY II based on the LD_{50} in both sexes.

Animals which died or which subsequently showed symptoms were generally normal through day 4; symptoms (decreased activity, pallor, piloerection, stains around nose) in some animals were observed only on the day of (or the day before) death. Some rats which were found dead had shown no previous signs of toxicity. Mortalities occurred 4-7 days after dosing. Necropsy findings in rats which died included pallor of the kidney, liver, lung, pancreas and spleen, and clotted and/or free blood in the thymus and/or thoracic cavity, consistent with the anticoagulant activity of brodifacoum. There were no consistent effects on body weight.

This acute oral LD_{50} study is classified as acceptable. This study <u>does</u> satisfy the guideline requirement for an acute oral study (81-1) in the rat for Brodifacoum Formulation Concentrate (0.25%)

2. In an acute dermal toxicity study (MRID No. 44021702), a group of five male and two groups each with five female young adult Alpk:APfSD (Wistar-derived) rats received a single 24-hour occluded dermal exposure to 2000 mg/kg undiluted Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%). At 24 hours the application site was cleansed with cotton swabs. In order to prevent ingestion of any residual material, rats were fitted with collars which were kept in place until day 4 for the males and first group of females, and throughout the observation period for the second group of females. The animals were observed for 14 days following removal of the occlusive dressings. 1/5 males and 2/10 females died on days 7-9 with symptoms consistent with anticoagulant activity; one of the dead females is reported to have chewed and partly removed the dressing.

Dermal LD_{50} Males > 2000 mg/kg
Females > 2000 mg/kg
Combined > 2000 mg/kg

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY III in terms of dermal toxicity potential, based on the dermal LD_{50} values in both sexes.

Among the survivors, one female showed bruising at the

application site on days 10-15. Necropsy findings (pallor of the brain, liver, lung, pancreas and/or spleen) for animals which were killed in extremis were consistent with anti-coagulant activity of brodifacoum. Survivors all gained weight.

This acute dermal LD_{50} study is classified as acceptable. This study does satisfy the guideline requirement for an acute dermal toxicity study (81-2) in the rat for Brodifacoum Formulation Concentrate (0.25%).

3. In a primary dermal irritation study (MRID No. 44021703), a group of six female young adult rabbits (New Zealand white), weights ranging from 3940-4290 g, each received a single 4-hour occluded dermal exposure to 0.5 ml of undiluted Brodifacoum Formulation Concentrate (0.25% a.i.), with scoring for dermal irritation within the first hour after removal of the occlusive wrap, and at 1, 2 and 3 days. There was slight edema only in one rabbit, and that was within one hour following exposure, but the test material stained the skin pink at the application sites, preventing assessment of erythema. However, subsequent histopathological examination of application and unexposed skin sites showed no inflammatory response associated with exposure to the test material.

   Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY IV in terms of dermal irritation potential, based on the lack of any significant irritation (slight edema observed in only one animal within one hour following exposure, and lack of inflammatory response observed in histopathological examination.

   This primary dermal irritation study is classified as acceptable. This study does satisfy the guideline requirement for a primary dermal irritation study (81-5) in the rabbit for Brodifacoum Formulation Concentrate (0.25%).

4. In a dermal sensitization study (MRID 44021704) with Brodifacoum Formulation Concentrate (0.25% a.i.), administered at challenge undiluted and as 30% and 10% w/v suspensions in deionized water, young adult Crl:(HA)BR male guinea pigs were tested using the method of Buehler.

   There were no indications of a sensitization reaction, although evaluation was complicated by pink staining at the application sites. Skin samples were examined histopathologically, with no indications of a significant inflammatory response. **In this study, Brodifacoum Formulation Concentrate (0.25% a.i.) is not a dermal sensitizer.**

   This study is classified as acceptable. It does satisfy the guideline requirement for a dermal sensitization study (81-6) in the guinea pig.

5. In the first part of a metabolism study (MRID 44021705) Brodifacoum, 3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one, radiochemical purity >98%, radiolabelled (^{14}C) in the benzene ring of the benzopyran, was administered to 3 previously bile-duct cannulated Crl:CD(SD)BR strain male rats as a single oral administration at a nominal dose level of 10 mg/kg body weight, well above the LD_{50} value of 0.3 mg/kg. The rats had been predosed with vitamin K_1 in their drinking water, but showed symptoms of anticoagulant toxicity before sacrifice at 48 hours. Bile, urine and feces were collected at pre-dose, 6, 12, 24, and 48 hr post-dose, and radioactivity was determined in these samples, as well as in the livers and residual carcasses. The metabolite profiles of ^{14}C-brodifacoum in bile and bile extracts were examined by chromatographic and spectroscopic techniques.

Total mean recovery of radioactivity was 102.9 ± 8.1%. Recovery from feces (presumably unabsorbed brodifacoum) was 36.11 ± 8.83%; from liver was 14.79 ± 0.41; from the residual carcass: 42.85 ± 5.06%. The mean from bile (all 3 animals) was 6.40 ± 5.45%, but one rat had poor bile flow, possibly from blockage in the cannula; the two remaining animals had a mean 9.53% of the label in bile.

The major (and only identified) metabolite of brodifacoum in bile was the glucuronide (attachment to the 4-hydroxy moiety of brodifacoum), which accounted for 39.43 to 77.28% of the total radioactivity in individual bile samples, while brodifacoum represented 0.00 to 24.95% of the total activity. Further characterization appeared to split the glucuronide peak into 2 components, and while the cis:trans ratio of parent material was 70:30, the ratio in the glucuronide was reversed (30:70). One unidentified metabolite (region 10) ranged from 1.59 to 21.7% total radiolabel.

In a second study (*in vitro* perfusion, also in MRID 44021705) the lower vena cava of a single male rat was ligated; the hepatic portal vein was then cannulated and the liver was cleared of blood and the bile duct cannulated. The liver was perfused, and, after equilibration, ^{14}C-brodifacoum, at a dose of 10 mg/kg, was added to the main perfusate reservoir; bile and perfusate were collected at pre-dose, 1 min (perfusate only), 1, 2, 3, 4 and 6 hr post-dose. The radioactivity present in bile, perfusate, terminal perfusate supernatant, supernatant filtrate and liver was determined.

In the second study there was 74.32% recovery after 6 hours, with 59% of the total in perfusate, and 15.19% in liver. Metabolite profiling was attempted, but no metabolites were identified. All radioactivity in the perfusate supernatant was bound to perfusate proteins, with no activity being measured in

the aqueous filtrate.

Although only one metabolite (the glucuronide) is identified, it is the parent compound which is of toxicological concern, and the registrant has adequately demonstrated in previously submitted studies (refer to MRIDs 00080235 and 42007502) that a high proportion of unmetabolized compound is retained, particularly in the liver. It is concluded that overall there is sufficient metabolism data (including excretion, distribution and amounts retained in different organs, retention half-life). This metabolism study in the rat then, when taken with previously submitted metabolism studies (in MRIDs 00080235 and 42007502) is classified as acceptable; and the combination of these studies is adequate to satisfy the 85-1 data (metabolism study) guideline requirement. It is noted that there are no registered uses of brodifacoum on food crops, and the registrant has submitted an acceptable antidotal study (in MRID 42007501).

cc: Briscoe/Rubis

012026

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 8/15/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. Clark Swentzel, Date 8/16/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Oral Toxicity - rat [81-1]

DP BARCODE: D227302

SUBMISSION CODE: S506631

P.C. CODE: 112701

TOX. CHEM. NO.: 114AAA

MRID NO.: 44021701

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (a.i. 0.259%)

SYNONYMS: 3-[3-(4'-Bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one; Talon

CITATION: Lees, D. & Leah, A. M. (1996). Brodifacoum Formulation Concentrate (0.25% w/w): Acute Oral Toxicity to the Rat. Zeneca Central Toxicology Laboratory, U.K. Report No. CTL/P/4672; Study No. AR5937, January 17, 1996. MRID 44021701. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID No. 44021701), groups of fasted, young Alpk:APfSD (Wistar-derived) rats, 5/sex were given a single oral dose of Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%) in deionized water at doses of 50, 200, or 500 mg/kg (males), and doses of 100, 150 or 200 mg/kg (females), and were subsequently observed for 14 days.

LD_{50} Males = 163 (95% C.I.: 97-275) mg/kg
Females = 152 (95% C.I.: 132-175) mg/kg
Combined = not reported

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY II based on the LD_{50} in both sexes.

Animals which died or which subsequently showed symptoms were generally normal through day 4; symptoms (decreased activity, pallor, piloerection, stains around nose) in some animals were

observed only on the day of (or the day before) death. Some rats which were found dead had shown no previous signs of toxicity. Mortalities occurred 4-7 days after dosing. Necropsy findings in rats which died included pallor of the kidney, liver, lung, pancreas and spleen, and clotted and/or free blood in the thymus and/or thoracic cavity, consistent with the anticoagulant activity of brodifacoum. There were no consistent effects on body weight.

This acute oral LD_{50} study is classified as acceptable. This study does satisfy the guideline requirement for an acute oral study (81-1) in the rat for Brodifacoum Formulation Concentrate (0.25%).

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 5), Data Confidentiality (p. 2) and Flagging criteria statements (p. 4) were provided.

A. **MATERIALS**:

1. Test Material: Brodifacoum formulation concentrate
   Description: a pink liquid
   Lot/Batch #: T42006
   Purity: 0.259% w/w brodifacoum
   CAS #: 56073-10-0
   Structure:

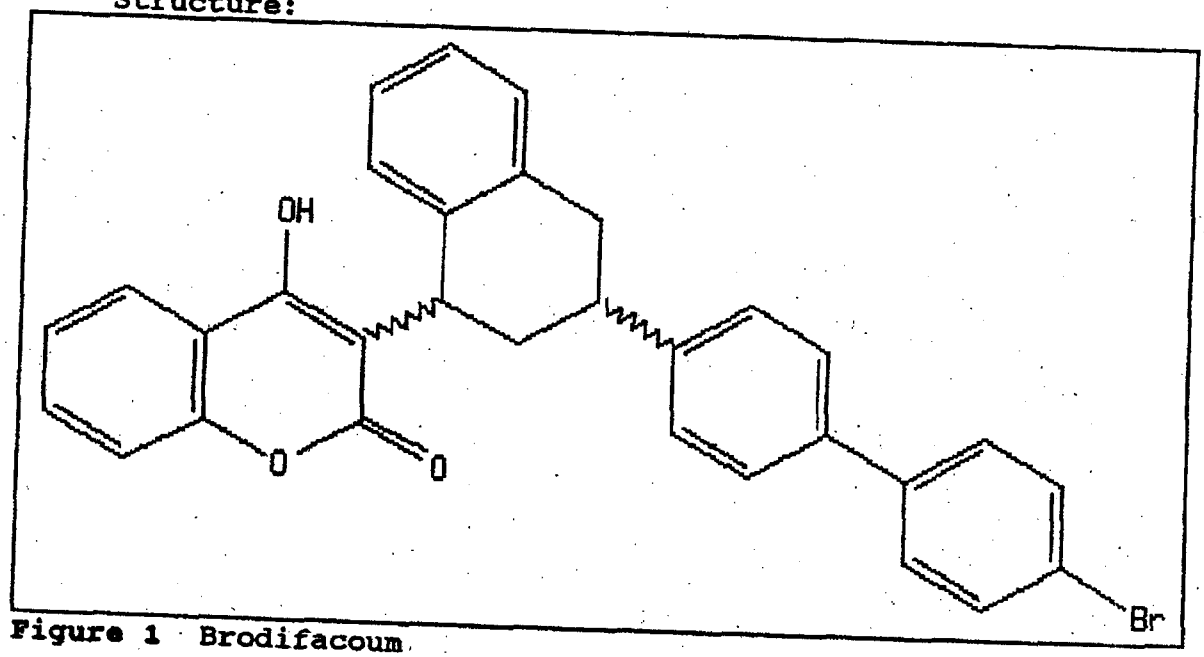

Figure 1 Brodifacoum

2. Vehicle:

From p. 10: "The formulation was...tested as preparations in

deionised water."

3. Test animals: Species: rat
Strain: Alpk:APfSD (Wistar-derived)
Age and weight at study initiation: "young adults"
males: 233-276 g; females: 194-253 g.
Source: from a colony maintained at Alderley Park.
Acclimation period: "a minimum of six days prior to the study."

B. STUDY DESIGN AND METHODS:

1. Animal assignment and treatment - Animals were assigned to to the test groups noted in table 1, following a preliminary study "in which a range of dose-levels was tested on small groups of animals. Based on information from this study the initial dose-level for the main study was selected as 200 mg/kg. Further dose-levels of 50 and 500 mg/kg for males and 100 and 150 mg/kg for females were added as the study progressed."

From information on p. 13 there was a pre-dose fast. "The rats were given a single oral dose of the formulation as a preparation in deionised water. A standard volume of 10 ml/kg was dosed to each animal and dose-levels were altered by varying the concentration of the dosed preparation... The preparations were administered by gavage..."

"The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made once daily up to day 15." Animals were weighed on day -1, 1 (the day of dosing), 3, 4, 8 and 15. Survivors were sacrificed and a gross necropsy was performed.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
50	0/5	-	0/5
100	-	0/5	0/5
150	-	2/5	2/5
200	3/5	5/5	8/10
500	5/5	-	5/5

Data from page 40 of the report.

2. Statistics - "The acute oral median lethal dose was estimated from the mortality data (the mortality data included animals that were killed in extremis) by logistic regression using nominal dose values. Confidence limits were calculated using a likelihood ratio interval..."

C. **RESULTS AND DISCUSSION:**

1. Mortality is given in table 1. Animals which died or showed symptoms were generally normal through day 4; mortalities occurred days 5-8.

2. Clinical observations - decreased activity, pallor, pilo-erection, stains around nose. Symptoms in some animals were seen only on the day of (or the day before) death, with some animals which were found dead having shown no previous signs of toxicity. Symptoms were consistent with anticoagulant activity of brodifacoum.

3. Body Weight - Most surviving animals gained weight during the observation period.

4. Necropsy - From p. 14: "A number of findings was observed, consistent with the known anticoagulant nature of the test material. These included pallor of the kidney, liver, lung, pancreas and spleen and clotted and/or free blood in the thymus and thoracic cavity."

The LD_{50} (95% C.I.) for males is 163 (97-275) mg/kg
females is 152 (132-175) mg/kg
combined is [not reported]

D. CLASSIFICATION: Acceptable. This 0.25% Brodifacoum formulation concentrate is in toxicity category II in terms of its oral hazard potential.

E. STUDY DEFICIENCIES: None. The study adequately defines the hazard potential of this formulation by the oral exposure route.

C12026

ONE-LINER

STUDY TYPE: Guideline: acute oral LD50 [§81-1]

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (0.259%)

PC CODE: 112701 CASWELL NO.: 114AAA

SYNONYM(S):

EPA MRID NO: 44021701

Testing Facility: Zeneca Central Toxicology Laboratory, U.K.

Study No.: CTL/P/4672

Report Issued: January 17, 1996

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID No. 44021701), groups of fasted, young Alpk:APfSD (Wistar-derived) rats, 5/sex were given a single oral dose of Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%) in deionized water at doses of 50, 200, or 500 mg/kg (males), and doses of 100, 150 or 200 mg/kg (females), and were subsequently observed for 14 days.

LD_{50} Males = 163 (95% C.I.: 97-275) mg/kg
Females = 152 (95% C.I.: 132-175) mg/kg
Combined = not reported

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY II based on the LD_{50} in both sexes.

Animals which died or which subsequently showed symptoms were generally normal through day 4; symptoms (decreased activity, pallor, piloerection, stains around nose) in some animals were observed only on the day of (or the day before) death. Some rats which were found dead had shown no previous signs of toxicity. Mortalities occurred 4-7 days after dosing. Necropsy findings in rats which died included pallor of the kidney, liver, lung, pancreas and spleen, and clotted and/or free blood in the thymus and/or thoracic cavity, consistent with the anticoagulant activity of brodifacoum. There were no consistent effects on body weight.

This acute oral LD_{50} study is classified as acceptable. This study does satisfy the guideline requirement for an acute oral study (81-1) in the rat for Brodifacoum Formulation Concentrate (0.25%).

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 8/5/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. Clark Swentzel, Date 8/16/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Dermal Toxicity - rat [81-2]

DP BARCODE: D227302

SUBMISSION CODE: S506631

P.C. CODE: 112701

TOX. CHEM. NO.: 114AAA

MRID NO.: 44021702

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (a.i. 0.259%)

SYNONYMS: 3-[3-(4'-Bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one; Talon

CITATION: Lees, D. & Leah, A. M. (1996). Brodifacoum Formulation Concentrate (0.25% w/w): Acute Dermal Toxicity to the Rat. Zeneca Central Toxicology Laboratory, U.K. Report No. CTL/P/4653; Study No. CR3227, January 17, 1996. MRID 44021702. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID No. 44021702), a group of five male and two groups each with five female young adult Alpk:APfSD (Wistar-derived) rats received a single 24-hour occluded dermal exposure to 2000 mg/kg undiluted Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%). At 24 hours the application site was cleansed with cotton swabs. In order to prevent ingestion of any residual material, rats were fitted with collars which were kept in place until day 4 for the males and first group of females, and throughout the observation period for the second group of females. The animals were observed for 14 days following removal of the occlusive dressings. 1/5 males and 2/10 females died on days 7-9 with symptoms consistent with anticoagulant activity; one of the dead females is reported to have chewed and partly removed the dressing.

Dermal LD_{50} Males > 2000 mg/kg
Females > 2000 mg/kg
Combined > 2000 mg/kg

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY III in terms of dermal toxicity potential, based on the dermal LD_{50} values in both sexes.

Among the survivors, one female showed bruising at the application site on days 10-15. Necropsy findings (pallor of the brain, liver, lung, pancreas and/or spleen) for animals which were killed in extremis were consistent with anti-coagulant activity of brodifacoum. Survivors all gained weight.

This acute dermal LD_{50} study is classified as acceptable. This study does satisfy the guideline requirement for an acute dermal toxicity study (81-2) in the rat for Brodifacoum Formulation Concentrate (0.25%).

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 5), Data Confidentiality (p. 2) and Flagging criteria statements (p. 4) were provided.

A. **MATERIALS:**

1. Test Material: Brodifacoum formulation concentrate
   Description: a pink liquid
   Lot/Batch #: T42006
   Purity: 0.259% w/w brodifacoum
   CAS #: 56073-10-0
   Structure:

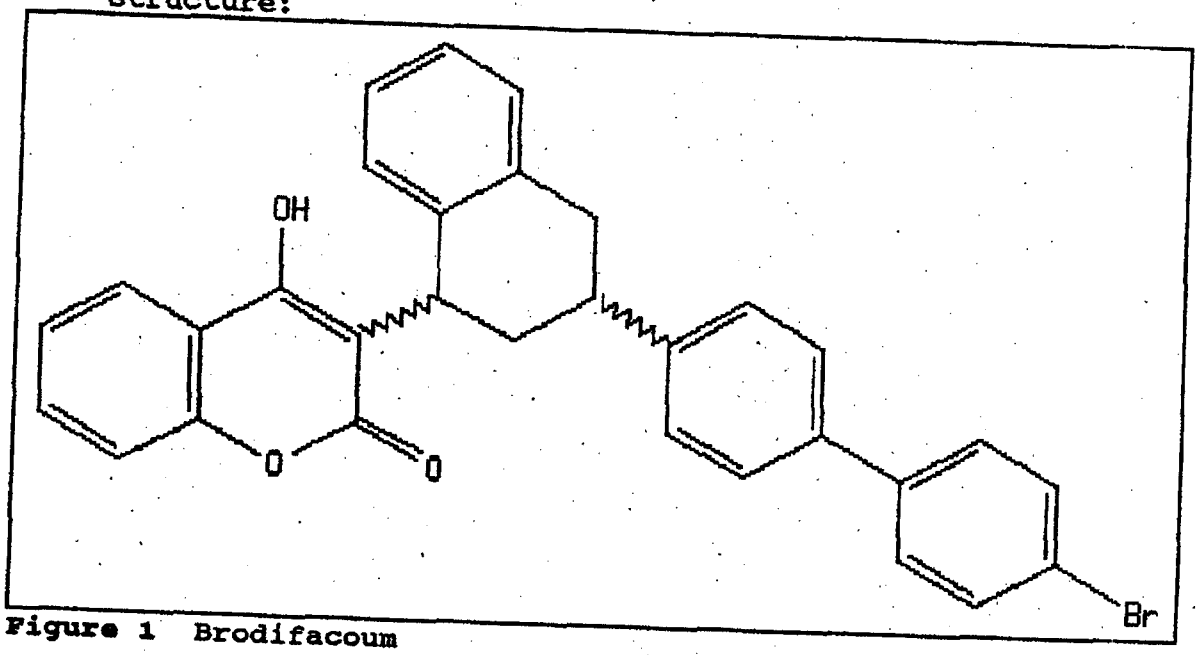

Figure 1 Brodifacoum

2. Application of Test Material:

From p. 12: "The undiluted formulation was spread evenly, using a 1 ml...disposable plastic syringe, onto the shorn back of each animal. A volume of 2 ml/kg was applied to each rat. The volume of the dose was calculated for each animal according to its weight at the time of dosing. The estimated amount applied per unit area of skin was 21.0 mg/cm^2 for male rats and 19.3 mg/cm^2 for female rats. The dosing preparation, covered by a 4-ply gauze patch, was applied to the shaven dorsum skin of each animal and kept in contact with the skin for 24 hours using an occlusive dressing."

3. Test animals: Species: rat
Strain: Alpk:APfSD (Wistar-derived)
Age and weight at study initiation: "young adults"
males: 253-275 g; females: 205-245 g.
Source: from a colony maintained at Alderley Park.
Acclimation period: "a minimum of six days prior to the study."

B. STUDY DESIGN AND METHODS:

1. Animal assignment and treatment - From p. 11: "The main study was preceded by a preliminary study (speculative dermal study) in which a range of dose-levels was tested on small groups of animals. Based on information from this study the dose-level for the main study was selected as 2000 mg/kg."

"The animals were observed for signs of systemic toxicity once within 2 hours of dosing and again between 4 and 7 hours after dosing. Subsequent observations were made once daily up to day 15." Animals were weighed on day -1, 1 (the day of dosing), 3, 4, 8 and 15. Survivors were sacrificed and a gross necropsy was performed.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
2000	1/5	2/10	3/15

Data from p. 46 of the report

2. Statistics - not done.

C. RESULTS AND DISCUSSION:

1. Mortality is given in table 1. Animals which died or showed symptoms were generally normal up to the day before or the

day of death. Mortalities occurred on days 7, 8, 9.

2. Clinical observations - decreased activity, hypothermia, piloerection, stains around nose. Symptoms were seen only on the day of (or the day before) death, with some animals which were found dead having shown no previous signs of toxicity. Symptoms were consistent with anticoagulant activity of brodifacoum. One of the surviving females (see report p. 29) showed "signs of bruising on right hand side of application site" from day 10 through day 15. The application site was generally stained pink (the color of the test material) on all animals. One of the females which died is noted on page 2 as having "parts of the gauze had been chewed and were found in the bottom of the cage."

3. Body Weight - Surviving animals gained weight during the observation period.

4. Necropsy - From p. 14: "Pallor of the brain, kidney, liver, pancreas and spleen was observed in animals which were killed in extremis. These findings are consistent with the known anticoagulant nature of the test material and are considered to be treatment-related."

The LD_{50} for males is > 2000 mg/kg
females is > 2000 mg/kg
combined is > 2000 mg/kg

D. CLASSIFICATION: Acceptable. This 0.25% Brodifacoum formulation concentrate is in toxicity category III in terms of its dermal toxicity hazard potential.

E. STUDY DEFICIENCIES: None. The study adequately defines the hazard potential of this formulation by the dermal exposure route.

ONE-LINER

STUDY TYPE: Guideline: acute dermal LD50 [§81-2]

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (0.259%)

PC CODE: 112701 CASWELL NO.: 114AAA

SYNONYM(S):

EPA MRID NO: 44021702

Testing Facility: Zeneca Central Toxicology Laboratory, U.K.

Study No.: CTL/P/4653

Report Issued: January 17, 1996

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID No. 44021702), a group of five male and two groups each with five female young adult Alpk:APfSD (Wistar-derived) rats received a single 24-hour occluded dermal exposure to 2000 mg/kg undiluted Brodifacoum Formulation Concentrate (active ingredient: Brodifacoum: label declaration 0.25%; analytical concentration 0.259%). At 24 hours the application site was cleansed with cotton swabs. In order to prevent ingestion of any residual material, rats were fitted with collars which were kept in place until day 4 for the males and first group of females, and throughout the observation period for the second group of females. The animals were observed for 14 days following removal of the occlusive dressings. 1/5 males and 2/10 females died on days 7-9 with symptoms consistent with anticoagulant activity; one of the dead females is reported to have chewed and partly removed the dressing.

Dermal LD_{50} Males > 2000 mg/kg
Females > 2000 mg/kg
Combined > 2000 mg/kg

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY III in terms of dermal toxicity potential, based on the dermal LD_{50} values in both sexes.

Among the survivors, one female showed bruising at the application site on days 10-15. Necropsy findings (pallor of the brain, liver, lung, pancreas and/or spleen) for animals which were killed in extremis were consistent with anti-coagulant activity of brodifacoum. Survivors all gained weight.

This acute dermal LD_{50} study is classified as acceptable. This study does satisfy the guideline requirement for an acute dermal toxicity study (81-2) in the rat for Brodifacoum Formulation Concentrate (0.25%).

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 8/5/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel, Date 8/8/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation - rabbit [81-5]

DP BARCODE: D227302

SUBMISSION CODE: S506631

P.C. CODE: 112701

TOX. CHEM. NO.: 114AAA

MRID NO.: 44021703

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (a.i. 0.259%)

SYNONYMS: 3-[3-(4'-Bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one; Talon

CITATION: Lees, D. (1996). Brodifacoum Formulation Concentrate (0.25% w/w): Skin Irritation to the Rabbit. Zeneca Central Toxicology Laboratory, U.K. Report No. CTL/P/4617; Study No. EB4362, February 12, 1996. MRID 44021703. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID No. 44021703), a group of six female young adult rabbits (New Zealand white), weights ranging from 3940-4290 g, each received a single 4-hour occluded dermal exposure to 0.5 ml of undiluted Brodifacoum Formulation Concentrate (0.25% a.i.), with scoring for dermal irritation within the first hour after removal of the occlusive wrap, and at 1, 2 and 3 days. There was slight edema only in one rabbit, and that was within one hour following exposure, but the test material stained the skin pink at the application sites, preventing assessment of erythema. However, subsequent histopathological examination of application and unexposed skin sites showed no inflammatory response associated with exposure to the test material.

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY IV in terms of dermal irritation potential, based on the lack of any significant irritation (slight edema observed in only one animal within one hour following exposure, and lack of inflammatory response observed in histopathological examination.

This primary dermal irritation study is classified as acceptable. This study does satisfy the guideline requirement for a primary dermal irritation study (81-5) in the rabbit for Brodifacoum Formulation Concentrate (0.25%).

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 5), Data Confidentiality (p. 2) and Flagging criteria statements (p. 4) were provided.

A. MATERIALS:

1. Test material: Brodifacoum formulation concentrate
   Description: a pink liquid
   Lot/Batch #: T42006
   Purity: 0.259% w/w brodifacoum
   CAS #: 56073-10-0
   Structure:

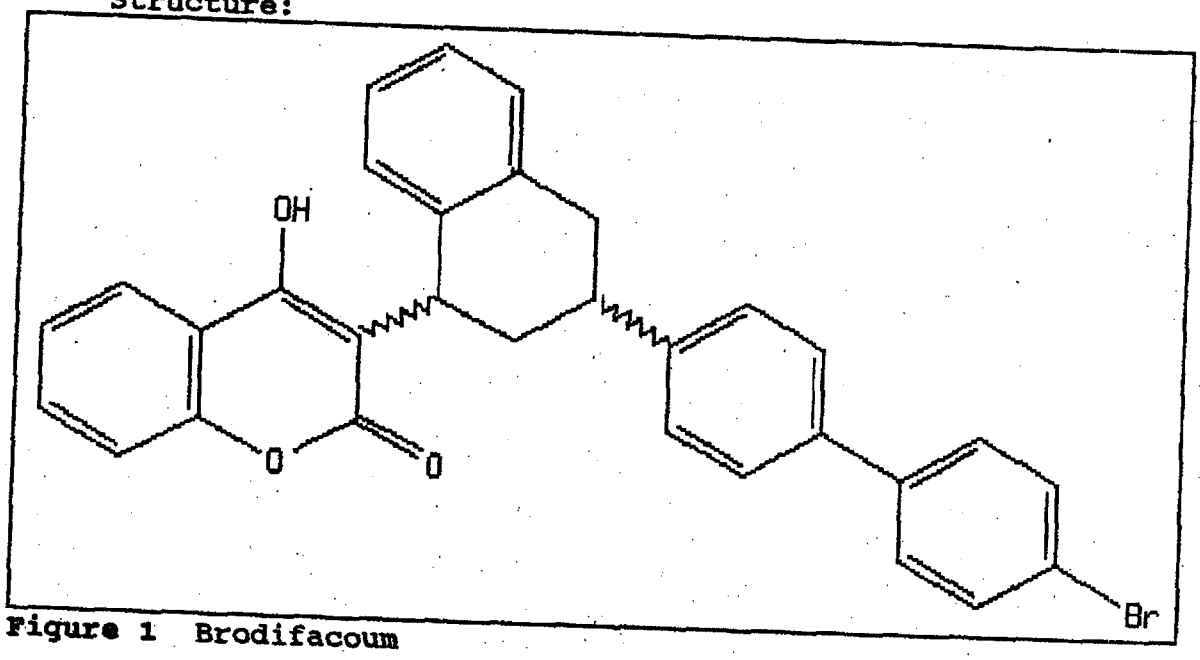

Figure 1 Brodifacoum

2. Test animals: Species: rabbit
   Strain: New Zealand White
   Age and weight at study initiation: "young adult"
   only females used: 3940-4290 g
   Source: Charles River UK Ltd., Kent, UK
   Acclimation period: "a minimum of six days prior to the study."

B. **STUDY DESIGN AND METHODS:**

1. Application of test material:

From p. 11: "...Approximately twenty-four hours before application of the test sample, the hair was removed...from an area approximately 7 cm x 13 cm on the left flank of each animal..."

"The test sample (0.5 ml) was applied, using a sterile polypropylene syringe, to the test site (approximate size 2.5 cm x 2.5 cm) on the left flank of each rabbit."

"The treated area was covered with a piece of surgical gauze...secured by two crossed strips of surgical tape... This was covered by a piece of impermeable rubber sheeting..."

"The dressings were left in position for approximately four hours. After this time each dressing was...removed and discarded. The application site was gently cleansed using...absorbent cotton wool soaked in clean warm water and then dried gently with clean tissue paper... The animal was then immediately collared in order to prevent oral ingestion of any residual test material on the application site following grooming."

2. Scoring:

From p. 12: "The Draize scale...was used to assess the degree of erythema and oedema at the application sites approximately 30-60 minutes, 1, 2 and 3 days after removal of the dressing... Any other signs of skin irritation were also noted."

C. **RESULTS AND DISCUSSION:**

Very slight, transient edema was observed in one animal only at 30-60 minutes after removal of the test substance. No edema was observed in other animals (all scores for edema were 0 at 1, 2 and 3 days).

The test material stained the skin pink which prevented accurate assessment of skin irritation potential. "On this basis, test and control skin samples were submitted for histopathological examination 3 days after decontamination."

On histopathologic examination, 2 control and 2 test site skin samples showed minimal focal acanthosis; otherwise there were no indications of dermal irritation.

D. **CLASSIFICATION**: Acceptable. This 0.25% Brodifacoum formulation concentrate is in toxicity category IV in terms of its dermal irritation hazard potential.

E. **STUDY DEFICIENCIES**: No significant deficiencies. The study adequately defines the a low dermal irritation hazard potential of this formulation.

012026

ONE-LINER

STUDY TYPE: Guideline: primary dermal irritation [§81-5]

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (0.259%)

PC CODE: 112701 CASWELL NO.: 114AAA

SYNONYM(S):

EPA MRID NO: 44021703

Testing Facility: Zeneca Central Toxicology Laboratory, U.K.

Study No.: CTL/P/4617

Report Issued: February 12, 1996

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID No. 44021703), a group of six female young adult rabbits (New Zealand white), weights ranging from 3940-4290 g, each received a single 4-hour occluded dermal exposure to 0.5 ml of undiluted Brodifacoum Formulation Concentrate (0.25% a.i.), with scoring for dermal irritation within the first hour after removal of the occlusive wrap, and at 1, 2 and 3 days. There was slight edema only in one rabbit, and that was within one hour following exposure, but the test material stained the skin pink at the application sites, preventing assessment of erythema. However, subsequent histopathological examination of application and unexposed skin sites showed no inflammatory response associated with exposure to the test material.

Brodifacoum Formulation Concentrate (0.25%) is in TOXICITY CATEGORY IV in terms of dermal irritation potential, based on the lack of any significant irritation (slight edema observed in only one animal within one hour following exposure, and lack of inflammatory response observed in histopathological examination.

This primary dermal irritation study is classified as acceptable. This study does satisfy the guideline requirement for a primary dermal irritation study (81-5) in the rabbit for Brodifacoum Formulation Concentrate (0.25%).

012026

BRODIFACOUM FORMULATION CONCENTRATE (0.25% w/w) Dermal Sensitization Study (81-6)

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 8/5/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. Clark Swentzel, Date 8/8/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Dermal Sensitization - guinea pig [81-6]

DP BARCODE: D227302

SUBMISSION CODE: S506631

P.C. CODE: 112701

TOX. CHEM. NO.: 114AAA

MRID NO.: 44021704

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (a.i. 0.259%)

SYNONYMS: 3-[3-(4'-Bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one; Talon

CITATION: Lees, D., Leah, A. M. (1996). Brodifacoum Formulation Concentrate (0.25% w/w): Skin Sensitisation to the Guinea Pig. Zeneca Central Toxicology Laboratory, U.K. Report No. CTL/P/4707; Study No. GG6391, February 14, 1996. MRID 44021704. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44021704) with Brodifacoum Formulation Concentrate (0.25% a.i.), administered at challenge undiluted and as 30% and 10% w/v suspensions in deionized water, young adult Crl:(HA)BR male guinea pigs were tested using the method of Buehler.

There were no indications of a sensitization reaction, although evaluation was complicated by pink staining at the application sites. Skin samples were examined histopathologically, with no indications of a significant inflammatory response. **In this study, Brodifacoum Formulation Concentrate (0.25% a.i.) is not a dermal sensitizer.**

This study is classified as acceptable. It does satisfy the guideline requirement for a dermal sensitization study (81-6) in the guinea pig.

COMPLIANCE: Signed and dated GLP (p. 3), Quality Assurance (p. 5), Data Confidentiality (p. 2) and Flagging criteria statements (p. 4) were provided.

A. **MATERIALS:**

1. Test material: Brodifacoum formulation concentrate
   Description: a pink liquid
   Lot/Batch #: T42006
   Purity: 0.259% w/w brodifacoum
   CAS #: 56073-10-0
   Structure:

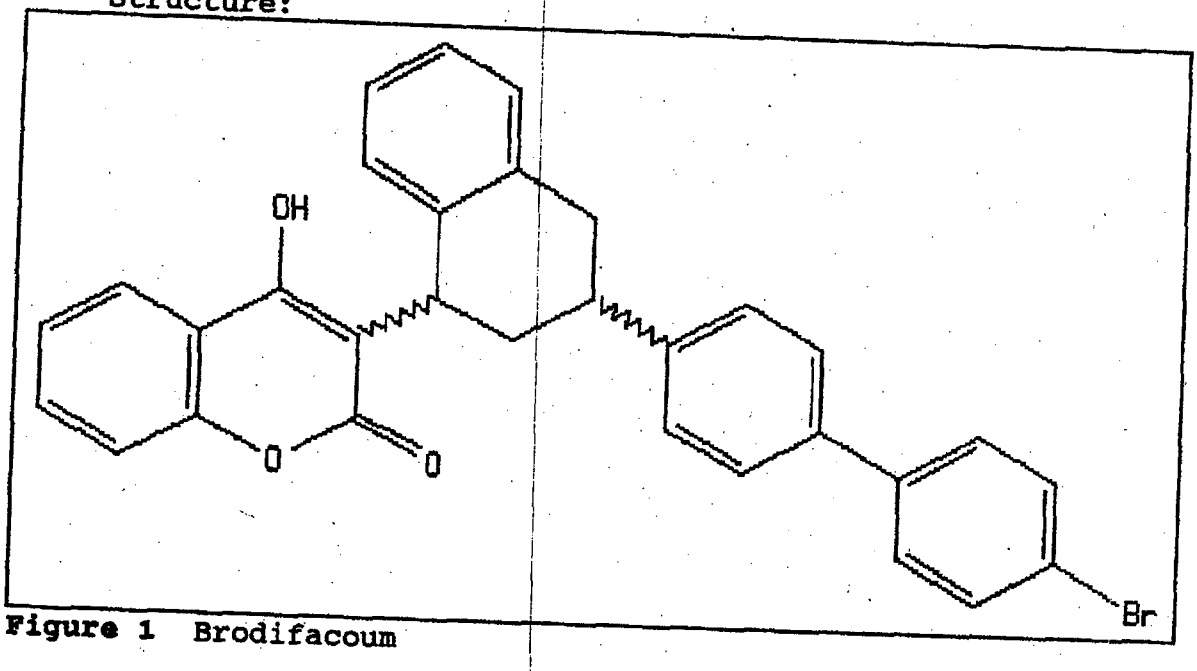

Figure 1 Brodifacoum

2. Vehicle and positive control:

   For induction (main study): 30% w/v suspension of test material (Brodifacoum formulation concentrate: 0.25%) in deionized water.
   Vehicle control: Deionized water
   Positive control: undiluted hexylcinnamaldehyde

3. Test animals: Species: guinea pigs
   Strain: Albino (Crl:(HA)BR)
   Age and weight at study initiation: "young adult"
   males (280-406 g) used in the main study
   females (304-382 g) used in the positive control study
   Source: Charles River UK Ltd., Kent, UK
   Acclimation period: "a minimum of six days prior to each study."

B. **STUDY DESIGN AND METHODS:**

1. In life dates - start: not reported end: not reported

2. Dose justification:

There were two preliminary or "sighting" studies. From p. 12: "The dose-levels for the induction and challenge stages of this study were determined by two sighting studies. The first of these studies was designed to establish potential dose-levels which gave acceptable levels of irritation. The second study was designed to ensure that the dose-levels selected for the main study were well tolerated in terms of systemic toxicity."

"The undiluted formulation and a 3% w/v preparation in deionised water were applied to each of two male guinea pigs, and 30% and 10% w/v preparations were applied to two male guinea pigs... Following application of the undiluted formulation the skin sites were stained pink by the formulation, obscuring [any possible?] erythema, however, there were no other signs of irritation. Following application of the 30% w/v preparation, the skin of one animal was stained and obscured and there were no signs of irritation in the other animal. There were no signs of irritation following application of the 10% or 3% w/v preparations..."

In the second sighting study (see p. 13) the test material was applied undiluted and as a 30% w/v preparation in induction dosages to separate groups, each consisting of 4 male guinea pigs. One male guinea pig was in the undiluted test material group was "killed humanely" on day 10 (24 hours after the third induction exposure) "due to severe bruising and haemorrhaging."

3. Animal assignment and treatment -

The Buehler method was used.

From p. 13: "A group of thirty male guinea pigs was used for the main study, twenty test and ten control. The study involved two main procedures, the induction of an immune response and a challenge of that response."

For induction: "An area approximately 5 cm x 5 cm on the scapular region of each animal was clipped free of hair...and treated with a topical application of 0.4 ml of either a 30% w/v preparation in deionised water (test-group) or deionised water only (control group). The freshly prepared sample...or deionised water:..was applied to a lint patch (approximate size 2 cm x 2 cm) which was covered with adhesive tape, and

held in place by an adhesive elastic bandage..."

"This occlusive dressing was left in place for approximately six hours. On removal of the dressings the animals were fitted with plastic collars, for approximately two days, in order to prevent oral ingestion during grooming. The induction procedure was repeated at the same site during the next two weeks, giving a total of three six-hour exposures. The interval between each exposure was 7 days. The irritation response was noted approximately 1 day after the removal of each patch and before application of each subsequent patch..."

"The animals were left untreated for two weeks after the final induction exposure, prior to challenge."

For challenge: "An area approximately 15 cm x 5 cm on both flanks of each animal was clipped free of hair with a pair of veterinary clippers. An occlusive dressing was prepared which consisted of four lint patches (approximate size 1 cm x 2 cm) stitched to a piece of rubber sheeting (approximately size 5 cm x 12 cm)."

"The undiluted formulation, preparations of the formulation [30% and 10%] in deionised water, or deionised water alone, were each applied to a lint patch. The dressing was placed on the shorn flank of the guinea pig so that the undiluted formulation was on the top left, the 30% w/v preparation was on the bottom left, the 10% w/v preparation was on the top right and deionised water was on the bottom right..."

"The patches were left in position for approximately six hours. The dressings were then...removed and discarded, and the animals were fitted with plastic collars until the following day."

"Skin sites were examined approximately 24 and 48 hours after removal of the dressings. The majority of the skin sites treated with the undiluted formulation, and several of those treated with the 30% w/v preparation, were stained and any erythematous reactions were obscured. Skin samples from all skin sites treated with these two concentrations were therefore submitted for histopathological examination..."

C. **RESULTS AND DISCUSSION:**

1. Induction reactions and duration - From p. 17: "The skin of the test animals was stained by the test material throughout the induction phase of the study, preventing the assessment of any slight erythema which may have been present. There were no overt signs of irritation in any test or control animals." All of the observations of the application sites

(see p. 25) 24 hours after each induction and immediately prior to the 2nd and 3rd induction applications are coded X:*OBS [from p. 20: stained by test sample + slight erythema would be obscured].

2. Challenge reactions and duration - From p. 17: "During the challenge phase of the study, the test material stained the skin on all animals treated with the undiluted formulation and the majority of those treated with the 30% w/v preparation, preventing the full assessment of erythema. Skin samples from these animals were therefore submitted for histopathological examination."

   From information on p. 28 all sites treated with undiluted test material, as well as all those treated with the 30% dilution, were stained at 24 hours after challenge, precluding a complete assessment of possible erythema (or lack thereof). However, at 48 hours after challenge, 2/20 sites which had been treated with undiluted test material as well as 17/20 of the sites which had been treated with the 30% dilution could be evaluated, and none of these sites showed any reaction. All sites which had been treated with the 10% dilution scored zero (no reaction) at both 24 and 48 hours.

   "Histopathological examination of skin sites treated with the undiluted formulation or a 30% preparation of the formulation revealed minor and insignificant instances of acanthosis and inflammatory cell infiltration in a small number of sections. There was no significant inflammatory response in either the test of control groups, indicating that the formulation did not elicit a skin sensitization response."

3. Positive control - It is stated (p. 10) that the positive control study (with hexylcinnamaldehyde) was carried out during January and February 1995. Sensitization responses (slight and/or moderate erythema; slight desquamation) were observed in most subjects at 24 hours after the 2nd and 3rd induction exposures; following challenge 13/20 previously exposed subjects showed slight ("scattered mild redness") to moderate ("moderate and diffuse redness") reactions at 24 hours, while 9/20 showed reactions at 48 hours.

3. Deficiencies - Normally, uncertainty in evaluating for a sensitization reaction because of the staining of the test sites from application of undiluted formulation or a 30% suspension in water, would be of some concern, particularly if the use pattern involved considerable and repeated dermal exposure. However, given the extreme toxicity of the active ingredient in this formulation, a staining reaction involving the skin and/or clothing could be construed as a desirable occurrence, since it would indicate exposure had taken place.

In any case, the histopathological findings indicate that this formulation does not cause a sensitization reaction, and so the study adequately defines the dermal sensitization potential of Brodifacoum Formulation Concentrate (0.25% w/w).

ONE-LINER

STUDY TYPE: Guideline: dermal sensitization [§81-6]

TEST MATERIAL (PURITY): Brodifacoum Formulation Concentrate (0.259%)

PC CODE: 112701 CASWELL NO.: 114AAA

SYNONYM(S):

EPA MRID NO: 44021704

Testing Facility: Zeneca Central Toxicology Laboratory, U.K.

Study No.: CTL/P/4707

Report Issued: February 14, 1996

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44021704) with Brodifacoum Formulation Concentrate (0.25% a.i.), administered at challenge undiluted and as 30% and 10% w/v suspensions in deionized water, young adult Crl:(HA)BR male guinea pigs were tested using the method of Buehler.

There were no indications of a sensitization reaction, although evaluation was complicated by pink staining at the application sites. Skin samples were examined histopathologically, with no indications of a significant inflammatory response. In this study, Brodifacoum Formulation Concentrate (0.25% a.i.) is not a dermal sensitizer.

This study is classified as acceptable. It does satisfy the guideline requirement for a dermal sensitization study (81-6) in the guinea pig.

012026

BRODIFACOUM

General Metabolism Study (85-1)

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 8/16/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. Clark Swentzel, Date 8/16/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Metabolism - [rat]; OPPTS 870.7485 [§85-1)]

DP BARCODE: D227302

SUBMISSION CODE: S506631

P.C. CODE: 112701

TOX. CHEM. NO.: 114AAA

MRID NO.: 44021705

TEST MATERIAL (PURITY): Radiolabelled Brodifacoum: radiochemical purity > 98%
Non-labelled Brodifacoum: 95.6%

SYNONYMS: 3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one; Talon

CITATION: Thornley, K. (1996). (^{14}C)-Brodifacoum: Metabolism in the rat. Corning Hazleton (Europe), North Yorkshire, England. Report No. 88/126-1011, February 8, 1996, MRID 44021705. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In the first part of a metabolism study (MRID 44021705) Brodifacoum, 3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one, radiochemical purity >98%, radiolabelled (^{14}C) in the benzene ring of the benzopyran, was administered to 3 previously bile-duct cannulated Crl:CD(SD)BR strain male rats as a single oral administration at a nominal dose level of 10 mg/kg body weight, well above the LD_{50} value of 0.3 mg/kg. The rats had been predosed with vitamin K_1 in their drinking water, but showed symptoms of anticoagulant toxicity before sacrifice at 48 hours. Bile, urine and feces were collected at pre-dose, 6, 12, 24, and 48 hr post-dose, and radioactivity was determined in these samples, as well as in the livers and residual carcasses. The metabolite profiles of ^{14}C-brodifacoum in bile and bile extracts were examined by chromatographic and spectroscopic techniques.

Total mean recovery of radioactivity was 102.9 ± 8.1%. Recovery from feces (presumably unabsorbed brodifacoum) was 36.11 ± 8.83%; from liver was 14.79 ± 0.41; from the residual carcass: 42.85 ±

5.06%. The mean from bile (all 3 animals) was 6.40 ± 5.45%, but one rat had poor bile flow, possibly from blockage in the cannula; the two remaining animals had a mean 9.53% of the label in bile.

The major (and only identified) metabolite of brodifacoum in bile was the glucuronide (attachment to the 4-hydroxy moiety of brodifacoum), which accounted for 39.43 to 77.28% of the total radioactivity in individual bile samples, while brodifacoum represented 0.00 to 24.95% of the total activity. Further characterization appeared to split the glucuronide peak into 2 components, and while the cis:trans ratio of parent material was 70:30, the ratio in the glucuronide was reversed (30:70). One unidentified metabolite (region 10) ranged from 1.59 to 21.7% total radiolabel.

In a second study (*in vitro* perfusion) the lower vena cava of a single male rat was ligated; the hepatic portal vein was then cannulated and the liver was cleared of blood and the bile duct cannulated. The liver was perfused, and, after equilibration, ^{14}C-brodifacoum, at a dose of 10 mg/kg, was added to the main perfusate reservoir; bile and perfusate were collected at pre-dose, 1 min (perfusate only), 1, 2, 3, 4 and 6 hr post-dose. The radioactivity present in bile, perfusate, terminal perfusate supernatant, supernatant filtrate and liver was determined.

In the second study there was 74.32% recovery after 6 hours, with 59% of the total in perfusate, and 15.19% in liver. Metabolite profiling was attempted, but no metabolites were identified. All radioactivity in the perfusate supernatant was bound to perfusate proteins, with no activity being measured in the aqueous filtrate.

Although only one metabolite (the glucuronide) is identified, it is the parent compound which is of toxicological concern, and the registrant has adequately demonstrated in previously submitted studies (refer to MRIDs 00080235 and 42007502) that a high proportion of unmetabolized compound is retained, particularly in the liver. It is concluded that there is sufficient metabolism data (including excretion, distribution and amounts retained in different organs, retention half-life). This metabolism study in the rat then, when taken with previously submitted metabolism studies (in MRIDs 00080235 and 42007502) is classified as acceptable; and the combination of these studies is adequate to satisfy the 85-1 data (metabolism study) guideline requirement. It is noted that there are no registered uses of brodifacoum on food crops, and the registrant has submitted an acceptable antidotal study (in MRID 42007501).

COMPLIANCE: Signed and dated GLP (p. 5), Quality Assurance (p. 9), Data Confidentiality (p. 3), and Flagging statements (p. 7) were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Compound:

[^{14}C]-Brodifacoum; 3-[3-(4'-bromo-[1,1'-biphenyl]-4-yl) 1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one, radiochemical purity >98%, radiolabelled (^{14}C) in the benzene ring of the benzopyran (received as two batches; refer to p. 30 of the report text). From p. 37: "The target isomer ratio of radiolabelled brodifacoum in the formulation was 60:40 cis:trans."
Radiochemical purity: >98% [following repurification flash column chromatography (batch 91-J13; original radiochemical purity 81.61%) or HPLC (batch 95-J6; original radiochemical purity 91.35%); measurement of final purity by HPLC
Lot/Batch numbers: 91-J13; 95-J6
Specific activity: 47.8 µCi/mg (batch 91-J13); 141.6 (batch 95-J6).

Non radioactive Brodifacoum
Purity: 95.6 % [determined by HPLC, GC or TLC]
Cis:trans ratio: 59.3:40.7
Lot/Batch No.: Y00052/035/001
Description: an "off white powder"
Contaminants: not described
CAS No.: 56073-10-0

2. Test animals:

Species: rat (males only)
Strain: Crl:CD(SD)BR
Age and weight at study initiation: 6-10 weeks
Source: Charles River (UK) Ltd., Kent
Housing: "groups of up to 5 per cage."
Diet: SQC Rat and Mouse Maintenance Diet No. 1 ad libitum
Water: "mains water" ad libitum
Environmental conditions:
Temperature: 19-23°C
Humidity: 40-70%
Air changes: a minimum of 10 per hour
Photoperiod: 12 hr light; 12 hr darkness
Acclimation period: approximately one week

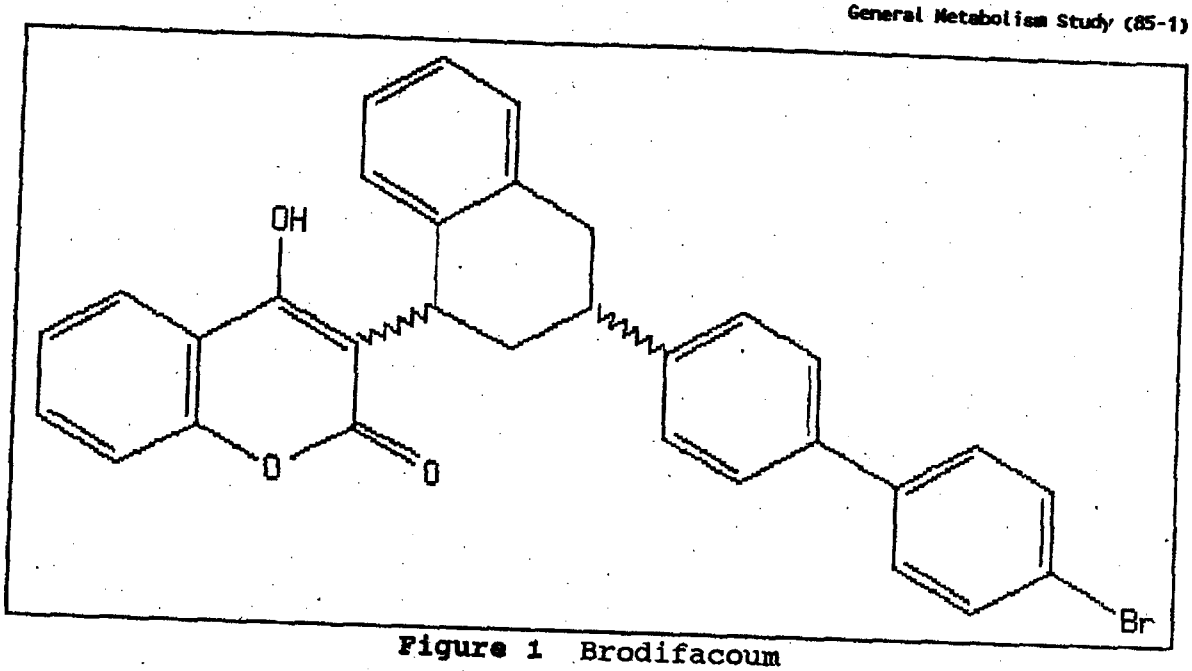

Figure 1 Brodifacoum

3. Preparation of dosing solutions:

From p. 36: "The test article for oral dosing was prepared as a solution in polyethylene glycol 600 (PEG 500) to provide a nominal dose volume of 5 mL/kg body weight. The vehicle for the test article administered to the animal via the circulating perfusate, during the *in vitro* liver perfusion, was polyethylene glycol 200 (PEG 200) at the same nominal dose volume as above."

II. **STUDY DESIGN AND METHODS - GROUP A:**

1. Group Arrangements

The animals were assigned randomly, from a group of 8 healthy male rats, to the test groups in table 1.

TABLE 1: Dosing groups for pharmacokinetic studies for Brodifacoum

Test Group	Dose of labelled material (mg/kg)	Number of males	Remarks
Oral dosing	10	3 (bile duct cannulated)	Bile, urine and feces collected pre-dose, 6, 12, 24 and 48 hr post-dose. Animals sacrificed at 48 hours; radio-activity in bile, urine, feces, liver and residual carcass was determined.
Oral & Perfusion	10 labelled (by cannulated perfusion) after an oral dose of 10 unlabelled	1*	Bile and perfusate collected at pre-dose, 1 min (perfusate only), 1, 2, 3, 4 and 6 hr post-dose. Radioactivity in bile, perfusate, terminal perfusate supernatant, supernatant filter and liver was determined.

*Selected from one of three rats orally dosed with 10 mg/kg unlabelled Brodifacoum on the basis of "body weight and greatest Vitamin K_1 consumption."

2. Dosing and sample collection - Group A:

a. Oral dosing

The test article was prepared as a solution in polyethylene glycol 600 to provide a nominal dose volume of 5 mL/kg. The [^{14}C]-Brodifacoum was administered orally by gavage at a dose level of 10 mg/kg body weight (= 50 µCi/rat).

"Approximately 24 h prior to surgery, drinking water was replaced with an aqueous solution of 1 mg/mL vitamin K_1... 2.5% Tween 80 was added to this solution to promote solubility of the vitamin K_1..."

"The animals were anaesthetised... The bile duct was cannulated through a mid-line abdominal incision and the cannula exteriorised dorsally. Following surgery, the animals were returned to individual metabolism cages and allowed to recover for approximately 24 h. Three animals were selected according to behaviour and

normal bile flow. [^{14}C]-Brodifacoum was administered at a nominal dose level of 10 mg/kg... The vessels used for collecting urine and faeces were surrounded by solid carbon dioxide. Bile was collected at room temperature..."

"Bile, urine and faeces were collected at the following intervals after dosing:

Bile and urine: Pre-dose, 6, 12, 24, and 48 h post-dose.

Faeces: Pre-dose, 6, 12, 24, and 48 h post-dose.

"Animals were...exsanguinated following CO_2 asphyxiation, and the liver was excised. The liver and carcass were retained and stored below -15°C until analysis."

b. Metabolite characterization - Group A:

"Metabolite profiles of [^{14}C]-brodifacoum and/or its metabolites in bile and extracts of bile were examined by various chromatographic (HPLC and TLC) and spectroscopic...techniques." "Analysis of neat bile was performed for all animals and time points in group A...

III. **RESULTS - GROUP A:**

1. Oral Dosing - Group A:

TABLE 2: Distribution of radioactivity in rat tissues at 48 hrs and excrement (collected 0-48 hrs) after oral administration of C^{14}-labeled Brodifacoum.

Collected Material or Tissue	Radioactivity recovery (%)
Feces	36.11 ± 8.83%
Urine	1.38 ± 0.41
Bile (all 3 animals)*	6.40 ± 5.45
Bile (without animal 103M)*	9.53 ± N.C.
Liver	14.79 ± 0.41
Residual Carcass	42.85 ± 5.06

*Poor bile flow observed in animal 103M; possibly due to blockage in cannula.
N.C. = not calculable (only two animals).
Data extracted from Tables 2, 3, 4, 5, 6 (p. 86, 87, 88, 89, 90).
Only a small percentage (mean of 1.4%) of administered radioactivity was recovered in cage washes.

From p. 48: "The mean recovery in biliary cannulated rats at study termination was 102.9 ± 8.1%."

"The cumulative total for all excreta was 45.3% of the administered dose. The faeces represented the primary route of excretion with 36.1 ± 8.8% of the dose being collected over 48 h the majority between 6 and 24 h (23.1%) and is likely to represent unabsorbed test material in these biliary cannulated rats."

"The majority (57.6%) of administered radioactivity had not been excreted by 48 h. The liver accounted for 14.7 ± 0.4% of the dose having a greater concentration of radioactivity, 35.9 ± 7.9 µg equivalents/g, than the residual carcass 4.8 ± 0.8 µg equivalents/g, which accounted for 42.9 ± 5.1% of the dose. The mean recovery in bile was 6.395 ± 5.454%, however, poor bile flow was observed in animal 103M which excreted only 0.126% over the study course, possibly due to a blockage in the cannula, as the volumes of bile sampled for this animal were low. Animals 101M and 102M excreted 10.05% and 9.005% (mean 9.528%) respectively, and were assumed to better reflect the excretion characteristics of this test substance.

"The urine represented a minor proportion of the administered dose with 1.4 ± 0.4% being recovered in this sample. Cage washings recovered a further 1.4%..., the radioactivity in both of these samples is attributed to faecal contamination in the cage during collection."

b. Metabolite characterization - Group A:

The major (and only identified) metabolite of brodifacoum was the glucuronide (From p. 2: the "mass difference of 176 is indicative of a glucuronide present on the 4-hydroxy moiety of the brodifacoum molecule. Hydroxylation would be required for the glucuronide to be present at any other position on the molecule, and a mass difference of 192 would have been observed.") From information on pages 95-99 the glucuronide represented from 39.43 to 77.28% of the total radioactivity in individual bile samples, while brodifacoum (sometimes reported as "cis" and "trans" forms) represented from 0.00 to 24.95% of the total radioactivity.

From p. 54: "Further characterization...appeared to split the glucuronide peak into two components, and

while the measured cis:trans ratio of brodifacoum was 70:30, the ratio of the glucuronide conjugate was reversed (30:70). These differences may possibly be indicating preferential isomeric metabolism, although chromatography effects, caused by the large molecular weight brodifacoum-glucuronide molecule, could account for the reversal of the cis and trans isomers in this chromatography system."

IV. **STUDY DESIGN AND METHODS - GROUP B:**

1. Group Arrangements

"Approximately 48 h prior to the initial dose of brodifacoum, 3 male rats were acclimatised in individual...cages." (Only one of these 3 rats was subsequently perfused).

a. *In vitro* rat liver perfusion - Group B

"The vehicle for the test article administered to the animal via the circulating perfusate, during the in vitro liver perfusion, was polyethylene glycol 200..." at the same nominal dose volume (5 mL/kg body weight) as in the oral dosing procedure.

"Approximately 24 h prior to surgery, drinking water was replaced with an aqueous solution of 1 mg/mL vitamin K_1... 2.5% Tween 80 was added to this solution to promote solubility of the vitamin K_1..."

"(unlabelled) Brodifacoum was administered orally, by gavage, to animals in group B, at a dose level of 10 mg/kg body weight. A further administration of [^{14}C]-brodifacoum was added directly into the main reservoir of the perfused animal, at a dose level of 10 mg/kg body weight corresponding to a nominal radioactive dose of 20 µCi...per animal..."

"A donor rat was selected (by body weight and greatest vitamin K_1 consumption), from the predose animals for surgery. The animal was anesthetized...and the peritoneal cavity opened along the mid-abdominal line ... A ligature was placed around the lower vena cava but not tied. The hepatic portal vein was then cannulated and a ligature used to secure the cannula in position. The liver was then cleared of blood by gently pumping pre-warmed heparinised Krebs-Ringer solution into the hepatic portal vein... The superior vena cava was then cannulated... The ligature on the

lower vena cava was then occluded and the bile duct cannulated prior to transferring the animal to the perfusion apparatus."

"Once both of the cannulae were attached to the apparatus, the clamp was removed, releasing perfusate from the head volume reservoir into the liver. The liver was allowed to equilibrate for approximately 30 minutes during which time control bile was collected, and the integrity of the preparation monitored by measuring liver perfusate flow."

Formulated [^{14}C]-brodifacoum at a dose rate of 10 mg/kg, was then added to the main reservoir prior to the collection of bile, and perfusate were collected at the following intervals...:

Bile: Pre-dose, 1, 2, 3, 4, and 6 h post-dose

Perfusate: Pre-dose, 1 min, 1, 2, 3, 4, and 6 h post-dose

"After each collection of perfusate (approximately 10 mL), an equal volume of fresh perfusate was added to the main reservoir to maintain volume."

"On termination of the experiment the liver was excised. The carcass and liver were retained and stored at < -15°C."

"The radioactivity present in bile, perfusate, terminal perfusate supernatant, supernatant filtrate and liver was determined."

b. Distribution of label in the perfusate - Group B

"An additional experiment was performed at 6 h, to investigate the distribution of radiolabelled material in the perfusate."

V. **RESULTS - GROUP B:**

1. *In vitro* Rat Liver Perfusion - Group B:

a. Recovery

From p. 49: "The recovered radioactivity from rat 201M, six hours after the addition of [^{14}C]-brodifacoum...was 74.3%. This value does not include radioactivity associated with the carcass, residual

perfusate in the apparatus, and perfusate that had leaked from the liver perfusion, hence a mass balance was not attempted."

"Elimination of [^{14}C]-brodifacoum and/or metabolites in the bile during the perfusion accounted for 0.1% of the administered dose at study termination (6 h). Radioanalysis of the liver accounted for 15.2% while the radioactivity associated with the recovered perfusate was 59.0%."

Table 3: Recovery of radioactivity from the liver perfusion 6 h following the introduction of [^{14}C]-brodifacoum at a nominal dose level of 10 mg/kg body weight to the perfusate - Group B

	Per cent of administered dose
SAMPLE	Animal 201M
Bile	0.134
Perfusate	59.00
Liver	15.19
TOTAL	74.32

Extracted from Table 8, p. 92.

From p. 52: "Metabolite profiling by HPLC was performed, however due to low levels of radioactivity no meaningful evaluation of chromatograms was possible, although there was some evidence for a radiolabelled component with a similar retention time to brodifacoum. No mass spectral identification was attempted."

b. Distribution of Radiolabelled Material in Perfusate:

"There were only marginal differences in the amount of radioactivity in the red blood cells (8.4 µg equivalents/g) and the perfusate supernatant (6.4 µg equivalents/g). Further analysis of the supernatant for protein binding of radioactive residues, indicated that all of the radioactivity in the perfusate supernatant was bound to perfusate proteins, with no activity being measured in the aqueous filtrate."

"Due to the low recovery of radioactivity in bile, the remaining animals were not used."

Table 4: Distribution of radioactivity in the perfusate 6 h following the introduction of [^{14}C]-brodifacoum at a nominal dose level of 10 mg/kg - Group B

	concentration µg/g
Red blood cells	8.371
Supernatant	6.388
Supernatant filtrate	0.006

Extracted from Table 9, p. 93.

VI. DISCUSSION

A. Overall Summary:

The stated objectives of this study were:

"To measure the rate of elimination and extent of metabolism of [^{14}C]-brodifacoum in bile following oral administration to the rat"

"To generate metabolites *in vitro*, by *in situ* rat liver perfusion with [^{14}C]-brodifacoum 24 h after a single oral dose of non-radiolabelled test article."

A major finding of this study is the identification of the brodifacoum-glucuronide conjugate as the major biliary metabolite of brodifacoum following oral dosage, although unchanged brodifacoum was also present in bile. The brodifacoum-glucuronide conjugate represented from 39.43 to 77.28% of the label in individual bile samples (in two of the three rats cumulative bile samples contained a mean of approximately 9.5% of the total label; in the third rat the value was considerably lower - 0.126% of the administered dose - and there may have been blockage of the bile duct).

A second finding was that a considerable portion of the administered dose (cumulative totals ranging from 30.04 to 46.24%, with a mean of 36.11%) was excreted in feces, presumably as unabsorbed material [the rat with the highest level of radioactivity in the feces - 46.24% - was the same one showing the lowest percent of administered dose - 0.126% - in the bile, presumably from blockage of the bile duct; but it is not certain that this was a cause-and-effect relationship]. In the one-liner from a previously reviewed rat metabolism study (CTL/P/462, dated 6/20/79; acc. nos. 250077, 245704 and 252894) the statement is made: "Following an oral dose of 0.25 mg/kg, brodifacoum is

almost completely absorbed and only very slowly excreted in the urine. The half life is estimated as 150-200 days with at least 50% of the retained radioactivity represented by the parent compound." The dose level of 10 mg/kg administered in this current study was well above the 0.25 mg/kg at which "almost complete" absorption occurred.

An additional finding, that while the measured cis:trans ratio of brodifacoum was 70:30, the ratio of the glucuronide conjugate appeared to be reversed (30:70), is also noted.

The occurrence of retention of brodifacoum by the liver has been previously reported (MRID 420075-02; approximately 11-12% of the dose was retained in the liver after 104 weeks in rats which had received doses of 0.02 or 0.15 mg/kg), although retention was "biphasic" at 0.35 mg/kg, with a half-life for elimination of approximately 4 days during the period 1-4 days after dosing and a half-life of 128 days in the period 28-84 days after dosing.

The major finding in the perfusate study was that nearly all the radioactivity in the perfusate supernatant appeared to be bound to perfusate proteins, with no activity being measured in the aqueous filtrate.

B. Study deficiencies:

The major deficiencies are the lack of identification of metabolites (other than the glucuronide) in the first study and the rather minimal amount of data (including lack of metabolite profiling) in the second study (in which only one rat was used). However, it is noted that brodifacoum has no registered food crop uses, and metabolism studies (except for antidotal data) are normally required only on a case-by case basis for non-food-use compounds.

It is also noted that the registrant has previously submitted other metabolism studies which have been reviewed, as well as an antidote study (in MRID 42007501) which has been reviewed and classified as acceptable.

The Tier 1 requirements for metabolism data state that a single low dose is required for each route of exposure and "the low dose should be non-toxic, but high enough to allow for metabolite identification in excreta." However, the oral LD50 value for brodifacoum is about 0.3 mg/kg; as indicated in the introduction (p. 27 of the report): "At dose levels of this magnitude...a significant proportion of the dose is bound to tissues in the animal and only

approximately 30% of a radiolabelled dose is eliminated in excreta, mainly in faeces. Only small amounts of metabolites can therefore be generated by dosing non-toxic amounts of brodifacoum, and extraction and identification of these from faeces would be likely to involve considerable technical problems..." This adequately justifies dosage at 10 mg/kg.

While the liver was the only organ for which radioactivity was measured in this study, the registrant has previously submitted a report (CTL/P/462, dated June 20, 1979; Acc. no. 245704) with the following findings (mostly reported in Caswell file document 003989, but with some modification by this reviewer after examination of the material in Acc. 245704 [= MRID 00080235]):

1) Three rats were orally dosed with labeled brodifacoum at 0.25 mg/kg, with termination after 10 days, and analyses (percentages ± S.D.) of abdominal fat (3.29 ± 0.36% of label), kidneys (0.78 ± 0.07%), heart (0.10 ± 0.00%), liver (22.84 ± 0.68%) and residual carcasses plus skin (50.82 ± 3.15%). The mean cumulative amounts (± S.D.) excreted in urine and feces were 1.31% (± 0.19) and 11.01% (± 1.29), respectively, and for bile was 1.36 (± 0.21)% (mean total label recovery of 91.51%).

"Analysis showed that 31.3% and 19.6% of the dose was present in the carcass and liver respectively as unchanged brodifacoum together with two other more polar components which were not identified. The biological half-life for the radioactive species in tissues was estimated to be 150-200 days."

2) Three rats received an oral dose of 0.25 mg/kg of labeled brodifacoum; mean percentages of dose present after 10 days were: pancreas: 2.33 (± 0.01)% ; spleen: 0.16 (± 0.02)%; and blood: 0.05 (± 0.01)%.

3) Three rats were orally dosed with labeled brodifacoum at 0.5 mg/kg and urine and feces were collected at 24-hour intervals for 5 days. The mean percent of dose excreted in the urine was 2.94% after 5 days, with most (total of 2.05%) excreted in the first 24 hours. In the feces, the cumulative mean percentage of total dose excreted after 5 days was 30.75%, with the highest excretion (14.95% of the total dose) on day one. One rat died on day three, one on day 4, and the remaining one died on day five. The following table gives the results from this group of rats:

Table 5. Excretion of radioactivity in the urine and feces of male rats given a single oral dose of ^{14}C-brodifacoum at 0.5 mg/kg.

Rat #	Route of excretion	% of dose excreted on day: 1	2	3	4	5	Total % excreted
44	Urine	1.81	0.40	0.17	-	-	2.38
	Feces	14.08	10.15	1.80	-	-	26.03
45	Urine	2.03	0.32	0.19	0.09	-	2.63
	Feces	16.47	10.94	5.01	0.98	-	33.40
46	Urine	2.31	0.46	0.30	0.29	0.47	3.83
	Feces	14.37	10.45	6.09	1.96	no feces	32.87

Rat 44 died on Day 3.
Rat 45 died on Day 4.
Rat 46 died on Day 5.

Data extracted from Table 2 (p. 96) of MRID 00080235.

4) Three rats were orally dosed with labeled brodifacoum at 1.5 mg/kg, and urine and feces were collected at 24-hour intervals for 5 days. The mean total percent of dose excreted in the urine was 2.81% after 5 days, with most (total of 2.01%) excreted on day one. In the feces, the total mean percent of dose excreted after 5 days was 42.56%, with the highest excretion (16.23% of the dose) occurring on day two. All 3 rats were dead by day 5.

Table 6. Excretion of radioactivity in the urine and feces of male rats given a single oral dose of ^{14}C-brodifacoum at 1.5 mg/kg.

Rat #	Route of excretion	% of dose excreted on day: 1	2	3	4	5	Total % excreted
47	Urine	1.86	00340	0.21	0.16	0.09	2.63
	Feces	15.55	12.85	9.63	5.82	no feces	43.85
48	Urine	1.99	0.35	0.24	0.08	-	2.66
	Feces	13.26	16.94	8.69	2.77	-	41.66
49	Urine	2.18	0.48	0.33	0.14	0.02	3.15
	Feces	14.14	18.89	6.42	2.74	no feces	42.19

Rat 47 was killed on Day 5.
Rat 48 died on Day 4.
Rat 49 died on Day 5.

Data extracted from Table 2 (p. 96) of MRID 00080235.

5) One rat was orally dosed with labeled brodifacoum at 0.25 mg/kg and expired air was collected for 48 hours. No radioactivity (presumably above background) was detected in expired air over this 48 hours.

6) The bile ducts of three rats were cannulated. After recovery, the rats were orally dosed with 0.25 mg/kg labeled brodifacoum, and the bile was collected at 24-hour intervals for 48 hours. According to the original review (Caswell document 003989): "The mean percent of dose excreted in the bile was 0.55 for the first 24 hours and 0.77 for the second with a total of 1.36." [This reviewer gets 1.32 by adding 0.55 and 0.77; actually, the statement should have been: "The mean percent of dose excreted in the bile was **0.59** for the first 24 hours and 0.77 for the second with a total of 1.36")].

7) Following an oral dose of 0.25 mg/kg labeled brodifacoum, autoradiograms of sagittal whole-body sections showed that 24 hours after administration, "the highest concentrations of radioactivity were present in the liver, pancreas and salivary glands. Radioactivity was also present in the gastric mucosa, intestinal mucosa, kidneys, adrenals, meninges, fat and skin. At 5 and 10 days post-dosing, the autoradiograms showed that the high levels of radioactivity persisted in [these] tissues...thus confirming the results obtained in the tissue retention studies."

In addition, the registrant has also submitted a metabolism study (in MRID 42007502; review in Caswell file document 010297) in which four groups of male rats were dosed according to the following regimen:

Table 7. Dosage regimen in the metabolism studies reported in MRID 42007502

Group	Study Number	Animal Numbers	Nominal Dose (mg/kg)	Nominal Dose (mBq/kg)
1	UR0172	1-21	0	0
2	UR0172	22-60	0.02	0.02
3	UR0172	61-120	0.15	0.15
4	UR0211	31-78	0.35	1.0

Table from p. 13 of MRID 42007502.

A summary of the findings of this study are given below (these are taken from Caswell document 010297):

1) Groups of 3 rats/one or more dosage level were

sacrificed at various times at up to and including week 104 (groups 2 and 3) and up to and including week 12 (group 4). At the time rats were sacrificed "samples of blood...were taken by cardiac puncture... The prothrombin time (PT) and kaolin-cephalin time (KCT) were measured. At the two non-toxic dose levels [groups 2 and 3] the clotting levels were unaffected during the study and were within the normal range (14-24 seconds for KCT and 12-15 seconds for PT). For group 4 rats, the prothrombin time reached a maximum of 148 seconds at 48 hours after dosage and was outside the normal range between 12 and 96 hours after dosing. After this time, the values were within the range for normal animals.

2) Excreta were collected for the 24-hour period prior to sacrifice times for groups 2 and 3. The highest levels of radioactivity in the feces were observed in rats sacrificed at 24 hours post-dosing (group 2: a mean of 5.22 ± 1.41% of the dose; group 3: 6.57 ± 0.55%). The only measurable amount of label in the urine (0.37 ± 0.02%) was observed in group 3 rats at 24-hours post-dosing.

3) The liver, kidneys, pancreas, salivary glands, and a 5-ml blood sample were taken at sacrifice, and the radioactivities of these samples were measured. In addition, for group 4 rats, a sample of abdominal fat was also taken. Measurements of the radioactivity of the frozen carcasses were also made on some occasions for group 3 animals. A considerable amount of the dose was retained in the liver, even at 104 weeks. From information on p. 28 of the original report, group 2 males still retained a mean of 11.78% of the dose in their livers at week 104; for group 3 males it was 11.74%; for group 4 males it was 21.24% at week 12 (week 13 values for groups 2 and 3 were 34.01% and 31.74%, respectively).

"For animals given <u>non-toxic</u> doses of brodifacoum (groups 2 and 3) the highest concentration of radioactivity was found in the liver one day after dosing. Smaller and somewhat similar concentrations of radioactivity were present in pancreas, salivary glands and kidneys at that time. Subsequent elimination of radioactivity from the liver of both groups of rats occurred at a similarly slow rate throughout the 2-year period... The half-life of elimination of radioactivity (excluding the day 1 value) was 350 days for both groups with a correlation coefficient of 0.96 for both values. The concentrations of radioactivity in salivary glands and kidney showed some fluctuation between day 1 and week 4 probably as a result of redistribution or metabolism and elimination of

brodifacoum. The concentration of radioactivity in the pancreas of rats in both groups increased steadily up to week 13 after dosing by which time it was greater than the corresponding value for liver" (although, as shown below in Tables 8 and 9, the total amounts of radioactivity in the liver were greater, because it is a larger organ).

Tables 8 and 9 show the proportion of radioactive label present in different organs at different times after dosage.

Table 8. The proportion of radioactivity expressed as a percentage of the dose in the tissues of male rats after the administration of a single oral dose of 14-C Brodifacoum (0.15 mg/kg) [from MRID42007502]

	% of dose									
Time after dosing	Liver Mean	SD	Kidneys Mean	SD	Sal. gland Mean	SD	Pancreas Mean	SD	Carcass Mean	SD
1 day	29.71	± 4.40	1.07	± 0.36	0.35	± 0.04	0.73	± 0.10	not done	
2 weeks	37.31	± 3.19	0.96	± 0.04	0.35	± 0.05	1.52	± 0.38	not done	
4 weeks	37.07	± 1.94	0.99	± 0.08	0.40	± 0.06	1.67	± 0.21	46.85	± 1.41
8 weeks	30.86	± 4.23	0.97	± 0.04	0.40	± 0.03	2.42	± 0.05	not done	
13 weeks	31.74	± 5.13	0.82	± 0.08	0.37	± 0.07	2.28	± 0.11	38.24	± 3.48
26 weeks	21.66	± 2.07	0.61	± 0.04	0.32	± 0.04	2.11	± 0.35	not done	
39 weeks	22.02	± 2.83	0.54	± 0.01	0.21	± 0.03	1.69	± 0.50	29.48	± 2.81
52 weeks	20.26	± 4.70	0.45	± 0.04	0.20	± 0.10	1.86	± 0.31	23.73	± 1.78
65 weeks	15.36	± 3.03	0.38	± 0.04	0.18	± 0.02	1.30	± 0.16	not done	
78 weeks	13.01	± 1.19	0.34	± 0.05	0.15	± 0.01	1.05	± 0.25	not done	
91 weeks	12.39	± 3.08	0.29	± 0.03	0.11	± 0.01	1.02	± 0.02	not done	
104 weeks	11.74	± 1.64	0.22	± 0.02	0.09	± 0.02	1.15	± 0.15	not done	

Data are the means from 3 rats/sacrifice time
Table from p. 30 of MRID 42007502.

Table 9. The proportion of radioactivity expressed as a percentage of the dose in the tissues of male rats after the administration of a single oral dose of 14-C Brodifacoum (0.35 mg/kg) [from MRID42007502]

	% of dose					
Time after dosing	Liver Mean	SD	Kidneys Mean	SD	Sal. gland Mean	SD	Pancreas Mean	SD
6 hours	19.62	0.27	0.71	0.02	0.12	0.05	0.27	0.16
12 hours	24.07	4.42	0.84	0.08	0.24	0.08	0.75	0.44
18 hours	28.04	2.81	0.89	0.03	0.24	0.12	1.55	0.21
24 hours	28.92	1.79	0.95	0.09	0.27	0.01	1.70	0.37
48 hours	26.47	1.75	0.82	0.04	0.26	0.03	1.73	0.19
72 hours	25.11	1.50	0.73	0.03	0.28	0.04	1.72	0.32
96 hours	25.05	1.71	0.73	0.03	0.26	0.03	1.97	0.24
8 days	22.52	3.28	0.68	0.08	0.29	0.03	1.82	0.52
14 days	23.89	2.89	0.61	0.06	0.24	0.03	1.85	0.34
28 days	23.47	1.21	0.59	0.02	0.26	0.04	1.73	0.12
56 days	23.00	0.09	0.59	0.02	0.26	0.02	2.00	0.14
84 days	21.24	3.19	0.55	0.06	0.25	0.03	2.02	0.40

Data are the means from 3 rats/sacrifice time
Table from p. 32 of MRID 42007502.

4) Aliquots (3-10 g) of pooled liver homogenates from rats in group 3 and group 4 (but not group 2) were analysed. Group 3 samples were exhaustively extracted with chloroform and group 4 samples with a mixture of dichloromethane:acetone (50:50 v/v). After centrifugation, the extracts were separately bulked and analysed for radioactivity when recoveries ranged between 97 and 117% of the radioactivity in the liver. After further processing, the samples underwent thin-layer chromatography.

A more polar component was present in the livers of group 4 rats (accounting for 11% and 9% of the radioactivity in the day 1 and day 14 extracts respectively) which could not be detected in the livers of group 3 animals. Two additional minor components (<1%) were also found. These data showed that at either dose level and irrespective of the time after dosing brodifacoum was the major component found in the liver and the cis:trans isomer ratio of the substance was not significantly altered. The more polar component had been shown to be present in the liver in previous studies.

* * * * *

Although only one metabolite (the glucuronide) is identified and characterized, it is the parent compound which is of toxicological concern, and the registrant has adequately demonstrated that an extremely high proportion of unmetabolized parent compound is retained, particularly in the liver. Overall, it is concluded that there is sufficient metabolism data (including excretion, distribution and amounts retained in different organs, as well as retention half-life) in the studies contained in MRIDs 00080235, 42007502 and 44021705 to adequately meet the 85-1 guideline requirement.

It is noted that there are no registered uses of brodifacoum on food crops, and the registrant has submitted an acceptable antidotal study (in MRID 42007501).