012085

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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OCT 25 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Developmental Toxicity (Rat and Rabbit) and Acute Oral LD50 Studies on Brodifacoum

FROM: Byron T. Backus, Ph.D., Toxicologist Byron T. Backus 10/16/96
Toxicology Branch 2
HED (7509C)

TO: John Redden
RCAB (7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 10/17/96
Section Head, Review Section II
Toxicology Branch 2
HED (7509C)

and

Yiannakis Ioannou, Ph.D., Acting Branch Chief J.M. Ioannou 10/21/96
Toxicology Branch 2
HED (7509C)

DP Barcode: D189478

Submission: S437475

Chemical: 112701 Brodifacoum

Action Requested: Review of studies for guidelines 81-1, MRID 42687501 and supplements to studies submitted for 83-3(a) and 83-3(b), MRID 42641902.

EXECUTIVE SUMMARIES:

1. In an acute oral toxicity study (MRID 42687501), groups of fasted young adult Wistar-derived Alpk:APfSD rats (5/sex) were given a single oral dose of brodifacoum (96.1%)/kg in PEG 300 (10 ml/kg b.w.) at doses of 0.25 and 0.5 mg/kg. In addition, one group of 5 males received a single oral dose of 0.35 mg/kg,

and one group of 5 females received a single oral dose of 0.75 mg/kg. The rats were observed for 14 days after dosing.

Oral LD_{50} Males = 0.418 mg/kg (95% C.L. = 0.350-0.500 mg/kg)
Females = 0.561 (95% C.L. = 0.472-0.667)
Combined = not reported
Brodifacoum (96.1%) is in TOXICITY CATEGORY I based on the LD_{50} values in both sexes.

There were no mortalities or signs of toxicity at 0.25 or 0.35 mg/kg. At 0.5 mg/kg 5/5 males and 1/5 females died (males all dead by day 7; the female dying on day 9); at 0.75 mg/kg 5/5 females were dead by day 6. Symptoms (pallor, subdued behavior, decreased activity, bruising and bleeding from the nose and/or rectum) and necropsy findings (free or clotted blood in the thoracic and/or abdominal cavity, kidney, esophagus and subcutaneous tissue) were consistent with the anticoagulant activity of brodifacoum.

This acute oral study is classified as **Acceptable (Guideline)**. This study does **satisfy** the guideline requirement for an acute oral study (§81-1) in the rat.

2. In a developmental toxicity study (MRIDs 00052443 and 40307202) brodifacoum (92.5%) was administered to 30 Alderley Park, Wistar-derived mated female rats/dose level by gavage in 10% v/v ethanol:water at dose levels of 0 (vehicle only), 0.001, 0.01 and 0.02 mg/kg/day from days 6 through 15 of gestation (as reported: in this study the morning on which spermatozoa were detected in vaginal smears was designated as day zero of gestation. In more recent studies the morning that sperm are detected is designated as day one of gestation).

There was blood in the uteri of one 0.01 and three 0.02 mg/kg females. This was considered to be possibly related to the administration of brodifacoum. There were no indications of any dose-related developmental effects associated with exposure to brodifacoum at doses up to and including 0.02 mg/kg/day. The dose level of 0.02 mg/kg/day is considered adequate, based on the occurrence of 100% mortality at a nominal value of 0.05 (analytical value of 0.35) mg/kg/day in a preliminary study, and blood measurements in a special study (Brodifacoum: Blood Kinetics Study in the Pregnant Rat, MRID 42641902).

The maternal NOEL is 0.001 mg brodifacoum/kg/day (based on the equivocal finding of blood in the uteri of one 0.01 and three 0.02 mg/kg females).

The developmental NOEL is 0.02 mg brodifacoum/kg/day (HDT).

This developmental toxicity study in the rat is classified as Acceptable (Guideline) (83-3a), and satisfies the guideline

requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat.

3. In a previous Agency review of a rat developmental toxicity study on brodifacoum the comment was made that: "Due to the cumulative toxicity of brodifacoum, maximum fetal exposure was not achieved until the end of and after the critical period of fetal development," and it was stated that: "It is recommended that the teratology studies be repeated...with dosing to start at the time of mating." In response, the registrant has conducted a special study (MRID 42641902) in which mixtures of unlabeled brodifacoum (98.7%) and radiolabelled brodifacoum (radiochemical purity >95%) were administered to Alderley Park, Wistar-derived mated female rats by gavage at nominal doses of 0.0125 mg/kg (Group A: 24 rats, starting on day 1 of gestation, with sacrifice by exsanguination of 3 rats on days 1, 3, 5, 7, 9, 11, 13, 16) and 0.02 mg/kg (Group B: 15 rats, starting on day 7, with sacrifice of 3 rats on days 7, 9, 11, 13 and 16). The test material was administered as a suspension in polyethylene glycol 600. Terminal blood samples were analyzed for brodifacoum levels.

   The following mean ng equivalents of brodifacoum/gram of maternal blood were observed: Group A (0.0125 mg/kg/day, days 0-16): day 1: 0.560; day 3: 0.924; day 5: 1.556; day 7: 1.809; day 9: 2.015; day 11: 2.795; day 13: 2.168; day 16: 3.396. Group B (0.02 mg/kg/day, days 7-16): day 7: 0.691; day 9: 1.362; day 11: 3.087; day 13: 2.427; day 16: 4.488.

   The relative proportions of mean blood brodifacoum levels in group B rats as compared to group A rats were the following: Day 7: 0.382; Day 9: 0.666; Day 11: 1.10; Day 13: 1.12; and Day 16: 1.32.

   In this study there was a steady increase of blood brodifacoum levels with continued dosage of both 0.0125 mg/kg/day and 0.02 mg/kg/day, consistent with findings of a previously reviewed metabolism study (MRID 00080235), in which three rats given a single oral dose of 0.25 mg labeled brodifacoum still retained a mean of 77.73% of the initial dose (mean total label recovery was 91.51%) after 10 days. The combination of high toxicity and body accumulation of brodifacoum would have eventually resulted in mortalities at these dosage levels at some time after 16 days.

   The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study, and the findings adequately justify the dosing schedule and doses used in the rat developmental toxicity study (MRID 00052443 and 40307202; summarization in MRID 92195013).

4. In a developmental toxicity study (MRIDs 00052442 and 40307201) brodifacoum (92.5%) was administered to 15 mated female Dutch rabbits/dose level by gavage in 5% v/v ethanol:water at dose levels of 0 (0.5% v/v aqueous Tween 80), 0 (5% v/v aqueous ethanol, the vehicle used with brodifacoum), 0.001, 0.002 and 0.005 mg brodifacoum/kg/day from days 6 through 18 of gestation (in this study gestation day 0 was the day of mating. In more recent studies it is designated as day one of gestation).

   Ten of the 15 rabbits receiving 0.005 mg/kg/day died or were humanely killed; all were found to have internal hemorrhage. Nine of these does had loss of blood (in some cases heavy) from the vagina. All of the implants of one doe (#46) in the 0.005 mg/kg/day group (killed on day 16) are reported to have had a hemorrhagic appearance, but otherwise there were no indications of any dose-related developmental or toxic effects associated with exposure to brodifacoum at doses up to and including 0.005 mg/kg/day. Because only three litters (and only 20 fetuses) were available from the 0.005 mg/kg/day group at 29 days (and taking into consideration the hemorrhagic appearance of the implants of #46), the NOEL for fetal toxicity is 0.002 mg/kg/day, and the LOEL is 0.005 mg/kg/day. The only possible indication of toxicity in the 0.002 mg/kg/day does was the occurrence of a small hemorrhage beneath the lid of one eye on gestation day 14 in one rabbit (#44) which was not pregnant, but a similar finding is not reported for the 0.005 mg/kg/day females.

   The maternal NOEL is 0.002 mg brodifacoum/kg/day. The LOEL is 0.005 mg/kg/day (based on 75% mortality associated with hemorrhage in pregnant females at this dose level). In addition, the prothrombin time was significantly increased at 0.005 mg/kg/day on day 20 relative to controls (to 26.5 [seconds?] from 14.5) in a preliminary range-finding study. The developmental toxicity NOEL is 0.002 mg/kg/day, as only 3 litters (with a total of 20 fetuses) were available at 0.005 mg/kg/day), and it is reported that all the implants from a 0.005 mg/kg/day doe which was killed on day 16 had a hemorrhagic appearance.

   This developmental toxicity study in the rabbit is classified as Acceptable (Guideline) (83-3b).

   cc: Frank Rubis

012085

[BRODIFACOUM] Developmental Study OPPTS 870.3700 (§83-3(a))

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 10/10/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. Clark Swentzel, Date 10/15/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - [rat]; OPPTS 870.3700 [§83-3 (a)]

DP BARCODE: D189478
P.C. CODE: 112701

SUBMISSION CODE: S437475
TOX. CHEM. NO.: 114AAA

TEST MATERIAL (PURITY): Brodifacoum (98.7%)

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

CITATIONS: Lappin, G.; Davies, D. (1992) Brodifacoum: Blood Kinetics Study in the Pregnant Rat: Study No. UR0394, Report No. CTL/P/3818, 11 November 1992. MRID 42641902. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In a previous Agency review of a rat developmental toxicity study on brodifacoum the comment was made that: "Due to the cumulative toxicity of brodifacoum, maximum fetal exposure was not achieved until the end of and after the critical period of fetal development," and it was stated that: "It is recommended that the teratology studies be repeated...with dosing to start at the time of mating" [Review by Zendzian dated Oct. 26, 1989, Caswell File Document #007588]. In response, the registrant has conducted a special study (MRID 42641902) in which mixtures of unlabeled brodifacoum (98.7%) and radiolabelled brodifacoum (radiochemical purity >95%) were administered to Alderley Park, Wistar-derived mated female rats by gavage at nominal doses of 0.0125 mg/kg (Group A: 24 rats, starting on day 1 of gestation, with sacrifice by exsanguination of 3 rats on days 1, 3, 5, 7, 9, 11, 13, 16) and 0.02 mg/kg (Group B: 15 rats, starting on day 7, with sacrifice of 3 rats on days 7, 9, 11, 13 and 16). The test material was administered as a suspension in polyethylene glycol 600. Terminal blood samples were analyzed for brodifacoum levels.

The following mean ng equivalents of brodifacoum/gram of maternal blood were observed: Group A (0.0125 mg/kg/day, days 0-16): day 1: 0.560; day 3: 0.924; day 5: 1.556; day 7: 1.809; day 9: 2.015; day 11: 2.795; day 13: 2.168; day 16: 3.396. Group B (0.02 mg/kg/day, days 7-16): day 7: 0.691; day 9: 1.362; day 11: 3.087;

day 13: 2.427; day 16: 4.488.

The relative proportions of mean blood brodifacoum levels in group B rats as compared to group A rats were the following: Day 7: 0.382; Day 9: 0.666; Day 11: 1.10; Day 13: 1.12; and Day 16: 1.32.

In this study there was a steady increase of blood brodifacoum levels with continued dosage of both 0.0125 mg/kg/day and 0.02 mg/kg/day, consistent with findings of a previously reviewed metabolism study (MRID 00080235), in which three rats given a single oral dose of 0.25 mg labeled brodifacoum still retained a mean of 77.73% of the initial dose (mean total label recovery was 91.51%) after 10 days. The combination of high toxicity and body accumulation of brodifacoum would have eventually resulted in mortalities at these dosage levels at some time after 16 days.

The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study, and the findings adequately justify the dosing schedule and doses used in the rat developmental toxicity study (MRID 00052443 and 40307202; summarization in MRID 92195013).

COMPLIANCE: A signed and dated GLP Statement is provided on p. 3, and a signed and dated Quality Assurance Statement is provided on p. 5. In addition, there is a Statement of Data Confidentiality Claim on p. 2, and an EPA Flagging Criteria Statement on p. 4.

I. MATERIALS AND METHODS

A. MATERIALS

1. Unlabelled Test Substance: brodifacoum
Description: an off-white powder
Batch #: not provided; obtained from Sorex Ltyd, Widnes, Cheshire, UK.
CTL Reference No.: Y00052/037
Purity: 98.7%
CAS No. 56073-10-0

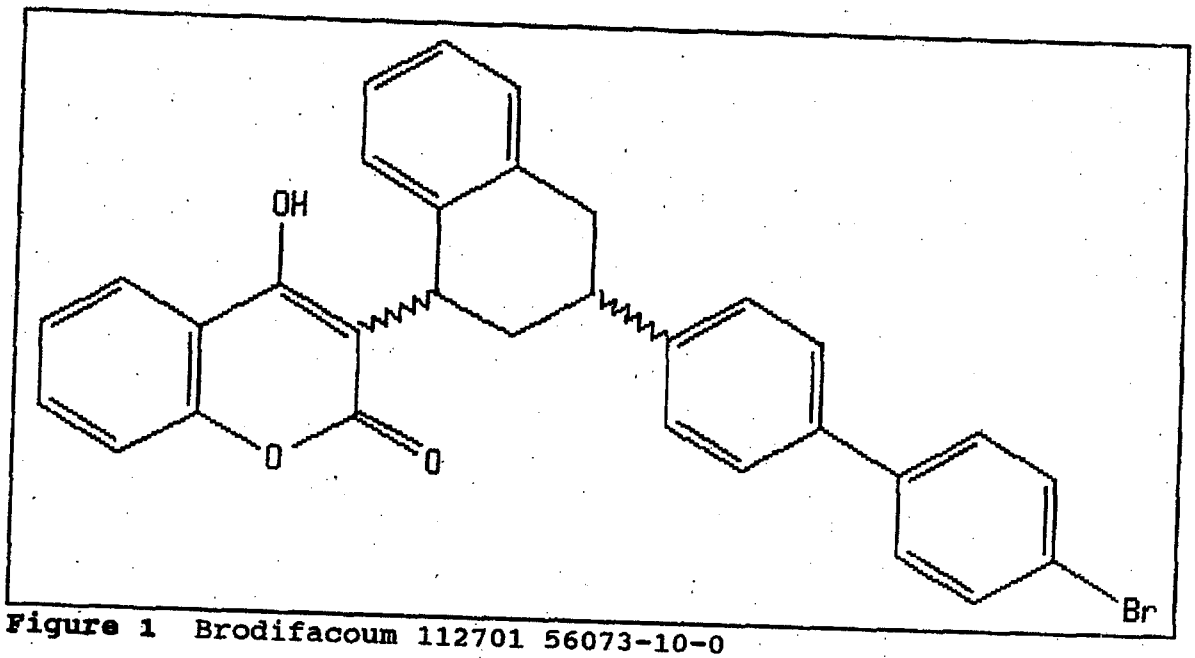

Figure 1 Brodifacoum 112701 56073-10-0

2. Radiolabelled Test Substance: [^{3}H]-labelled brodifacoum
Specific activity: 1.04 GBq/μmol
Radiochemical purity: >95% following repurification
CTL Reference No.: Y00052/036

3. Vehicle: Polyethylene glycol (PEG 600)

4. Test animals: Species: rat
Strain: Alderley Park Wistar-derived
Age at mating: not reported
Weight at start of dosing: 210 - 344 g
Source: Colony maintained at Alderley Park; supplied as mated animals.
Housing: Individually in stock rat cages
Diet: Pelleted Portion Combined Diet (PCD) suplied by Special Diet Services Ltd, Stepfield, Witham, UK ad libitum
Water: tap water ad libitum
Environmental conditions:
Temperature: 20-25°C
Relative humidity: 44-62%
Air changes: not stated
Photoperiod: 12 hrs light/ 12 hrs dark
Acclimation period: Not applicable because the females were mated at the breeding unit and then were sent to the laboratory.

B. PROCEDURES AND STUDY DESIGN

1. In life dates - From p. 12: "This study was performed between June 1992 and September 1992."

2. Mating: Virgin females were paired overnight with unrelated males of the same strain. On the following morning, vaginal smears from the females were examined for the presence of sperm. The day when sperm were detected was designated Day 1 of gestation and on this same day successfully mated females were delivered to the experimental unit at the Central Toxicology Laboratory..

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1.

Table 1. Animal assignment.

Animal Number	Dose Level (mg/kg/day)	Dosing Period (Days of Gestation)	Accumulated Dose (mg/kg)
1 - 3	0.0125	1	0.0125
4 - 6	0.0125	1 - 3	0.0375
7 - 9	0.0125	1 - 5	0.0625
10 - 12	0.0125	1 - 7	0.0875
13 - 15	0.0125	1 - 9	0.1125
16 - 18	0.0125	1 - 11	0.1375
19 - 21	0.0125	1 - 13	0.1625
22 - 24	0.0125	1 - 16	0.2
25 - 27	0.02	7	0.02
28 - 30	0.02	7 - 9	0.06
31 - 33	0.02	7 - 11	0.1
34 - 36	0.02	7 - 13	0.14
37 - 39	0.02	7 - 16	0.2

Data extracted from Table 1, page 16 of MRID 42641902.

4. Dose selection rationale: From p. 11: "The 0.02 mg/kg dose level administered between days 7 and 16 of gestation was chosen as it was the same as that used on the previous teratology study... Brodifacoum is known to accumulate in the rat and repeat administration will lead the blood level rising to a toxic concentration, causing death. The 0.0125 mg/kg dose level was selected therefore, in order that the total dose administered between days 1 and 16 of gestation was the same as the total dose administered between days 7 and 16 of gestation using the higher dose level. This was the highest dose which could be given over the respective periods for each dose group without causing toxic effects."

5. Dosage preparation and analysis:

From p. 13: "Three dosing solutions were prepared. Dosing solution 1, 0.0125 mg/kg was dosed between days 1 and 16 of gestation. This dosing solution was prepared as two separate batches (1a and 1b) as, initially, an insufficient quantity of this

preparation was made. Dosing solution 1a was administered up to day 11 of gestation, and 1b administered thereafter, for this dose group. Dosing solution 2, 0.02 mg/kg was dosed between days 7 and 16 of gestation." Doses were by gavage, with the material in PEG 600, at either 0.0125 mg/kg (1.5 MBq/kg) or 0.02 mg/kg (2MBq/kg). In the original rat developmental toxicity study (MRIDs 00052443 and 40307202) the vehicle used was 10% ethanol:90% water (v/v).

"Aliquots of the three dose preparations were diluted 10 times with acetonitrile and 20 µL injected into the HPLC system. Two minute fractions of the eluent were collected... The collected fractions were monitored for radioactivity content..."

Stability Analysis: "After a period of 30 days, dose preparation 1b was reanalysed..."

Results:

From p. 19: "HPLC column recoveries were close to 100%."

For stability analysis: "After 30 days the dose preparation showed a slight reduction of radiochemical purity to 91.7%."

6. Dose administration:

From p. 15: "Animals each received daily, oral, bodyweight-dependent doses of the [^{3}H]-brodifacoum in PEG 600 (2 ml/kg) by gavage. This dose rate was equivalent to a nominal dose of either 0.0125 mg/kg (1.5 MBq/kg) or 0.02 mg/kg (2 MBq/kg() respectively... All doses were administered by weight using a 2 ml gas tight syringe...fitted with a stainless steel gavage. The amount of radiolabelled dose given to each rat was determined by weighing the syringe and gavage assembly prior to and immediately after dosing..."

II. RESULTS

A. OBSERVATIONS

1. Maternal Observations and Evaluations: There are no indications within the text of this report as to the frequency of maternal observations. The statement is made (p. 17) that: "The uterus of each animal was

examined at termination. Pregnancy status was not determined prior to day 6 as this was pre-implantation. The uterus of each animal was removed and the left and right horn examined visually for implantation sites, the presence of which confirmed pregnancy."

Results: The statement is made (p. 21) that: "There were no indications of maternal toxicity for the duration of the study." Four of the 39 animals were not pregnant (and blood radioactivity measurements from non-pregnant and discarded animals were not used to obtain the mean equivalent concentrations of brodifacoum/g of rat blood shown in table 2 of this DER).

2. Measurement of Radioactivity in Blood: From p. 17: "...200 mg of blood, in duplicate, was oxidised... The [^{3}H]-water generated from the oxidation of the samples was flushed through the system with steam and mixed with scintillant (Monophase S). Samples were monitored for radioactivity content... Compensation was made for the efficiency of oxidation of test samples relative to [^{3}H] standard oxidation efficiencies which were determined at regular intervals. Background levels of radioactivity were determined by running blanks...and were deducted from the DPM values obtained from [the] scintillation counter. They ranged from 60 to 163 dpm."

From p. 20: "The limit of detection...of radioactivity measurement during this study was taken as 50 disintegrations per minute (dpm) per sample."

"For animals dosed with dose preparations 1a and 1b (0.0125 mg/kg) the limit of detection was 0.016 ng equivalents of brodifacoum per g of blood. For dose preparation 2 (0.02 mg/kg) the limit of detection was 0.019 ng equivalents of brodifacoum per g of blood. These values are based on a sample size of 0.2 g for all determinations."

Results: The following are the mean ng equivalents of brodifacoum/gram of rat blood:

Table 2. Mean concentrations of brodifacoum/gram of rat blood

Day	Cumulative Dose (in mg/kg) of Brodifacoum in Group A (0.0125 mg/kg/day) rats	Mean ng equivalents of Brodifacoum/gram of maternal blood in Group A (0.0125 mg/kg/day) rats	Cumulative Dose (in mg/kg) of Brodifacoum in Group B (0.02 mg/kg/day) rats.	mean ng equivalents of Brodifacoum/gram of maternal blood in Group B (0.02 mg/kg/day) rats
1	0.0125	0.560	-	-
3	0.0375	0.924	-	-
5	0.0625	1.556	-	-
7	0.0875	1.809	0.020	0.691 **
9	0.1125	2.015	0.060	1.362
11	0.1375	2.795	0.100	3.087
13	0.1625	2.168	0.140	2.427
16	0.2000	3.396	0.200	4.488

** Statistically significantly different at the 1% level by Student's t-test (two sided) from Group A on the same day of gestation.

Data extracted from Table 2 (p. 26) of MRID 42641902.

From information on pages 29-30, inclusion of data from non-pregnant animals would not have significantly changed the values given above (group A: day 5: change from 1.556 to 1.512; day 9: 2.015 to 2.041; day 11: 2.795 to 2.938; group B: day 16: 4.488 to 3.949).

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: It is stated (p. 22) that: "Radioactivity levels from day 10 to day 17 of gestation...were not significantly different for the two dose levels..."

"The achieved blood levels of brodifacoum using the two dosing regimes were therefore, not significantly different from at least day 10 onwards. They were thus equivalent for most of the dosing period from day 7 to 16 of gestation and hence for most of the period of major organogenesis."

"It has been the experience of this Laboratory in investigations into the timing of effects of known teratogens, for example by using "window dosing" i.e.

2-3 day periods of dosing during pregnancy, that days 10-12 of gestation are usually the most critical times for the production of structural abnormalities in the rat. The achieved blood levels of brodifacoum using the two dosing regimes of days 1-16 and days 7-16 of pregnancy were similar from about day 10 onwards and thus also similar both during this particularly sensitive period and the remainder of the period of major organogenesis."

"It is concluded that there were no significant differences between the groups in the foetal exposure to brodifacoum."

B. REVIEWER'S DISCUSSION

We can accept the findings of this study as justification for both the highest dosage and scheduling of the rat developmental toxicity study for brodifacoum (MRIDs 00052443 and 40307202). However, it is noted that there is a statement made in the text of this report (p. 11: "The 0.0125 mg/kg dose level was selected therefore, in order that the total dose administered between days 1 and 16 of gestation was the same as the total dose administered between days 7 and 16 of gestation using the higher dose level. **This was the highest possible dose which could be given over the respective periods for each dose group, without causing toxic effects.**") that does not seem to be completely supported by the data which the Agency has received for this chemical.

C. STUDY DEFICIENCIES AND ITS ADEQUACY: This study was conducted in response to a comment by Dr. Zendzian (Oct. 26, 1989; Caswell file document 007588) in a previous review of the study in MRIDs 00052443 and 40307202. In that review, the statement was made:

"Due to the cumulative toxicity of brodifacoum, maximum fetal exposure was not achieved until the end of and after the critical period of fetal development."

The study adequately addresses the comment by Dr. Zendzian.

The study is classified as Acceptable (Nonguideline) as it is not a required guideline study. It is acceptable for the purposes for which it was intended as a special study, and the findings adequately justify the dosing schedule and doses used in the rat developmental toxicity study (MRID 00052443 and 40307202; summarization in MRID 92195013).

012085

[BRODIFACOUM]

Acute Oral Study (81-1)

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 10/4/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. C. Swentzel, Date 10/15/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity [rat] OPPTS 870.1100 [§81-1];

DP BARCODE: D189478
P.C. CODE: 112701

SUBMISSION CODE: S437475
TOX. CHEM. NO.: 114AAA

TEST MATERIAL (PURITY): Brodifacoum (96.1%)

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

CITATIONS: Duerden, L; Brodifacoum: Acute Oral Toxicity to the Rat: ZENECA Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study No. AR5481, Report No. CTL/P/3918, 22 January 1993. MRID 42687501. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 42687501), groups of fasted young adult Wistar-derived Alpk:APfSD rats (5/sex) were given a single oral dose of brodifacoum (96.1%)/kg in PEG 300 (10 ml/kg b.w.) at doses of 0.25 and 0.5 mg/kg. In addition, one group of 5 males received a single oral dose of 0.35 mg/kg, and one group of 5 females received a single oral dose of 0.75 mg/kg. The rats were observed for 14 days after dosing.

Oral LD_{50} Males = 0.418 mg/kg (95% C.L. = 0.350-0.500 mg/kg)
Females = 0.561 (95% C.L. = 0.472-0.667)
Combined = not reported
Brodifacoum (96.1%) is in TOXICITY CATEGORY I based on the LD_{50} values in both sexes.

There were no mortalities or signs of toxicity at 0.25 or 0.35 mg/kg. At 0.5 mg/kg 5/5 males and 1/5 females died (males all dead by day 7; the female dying on day 9); at 0.75 mg/kg 5/5 females were dead by day 6. Symptoms (pallor, subdued behavior, decreased activity, bruising and bleeding from the nose and/or rectum) and necropsy findings (free or clotted blood in the thoracic and/or abdominal cavity, kidney, esophagus and subcutaneous tissue) were consistent with the anticoagulant activity of brodifacoum.

This acute oral study is classified as **Acceptable (Guideline)**. This study does **satisfy** the guideline requirement for an acute oral study (§81-1) in the rat.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: brodifacoum, 96.1%
   Description: an off-white solid
   Reference: P3/D7534/53
   CTL Reference No.: Y00052/038
   Purity: 96.1%
   CAS No. 56073-10-0

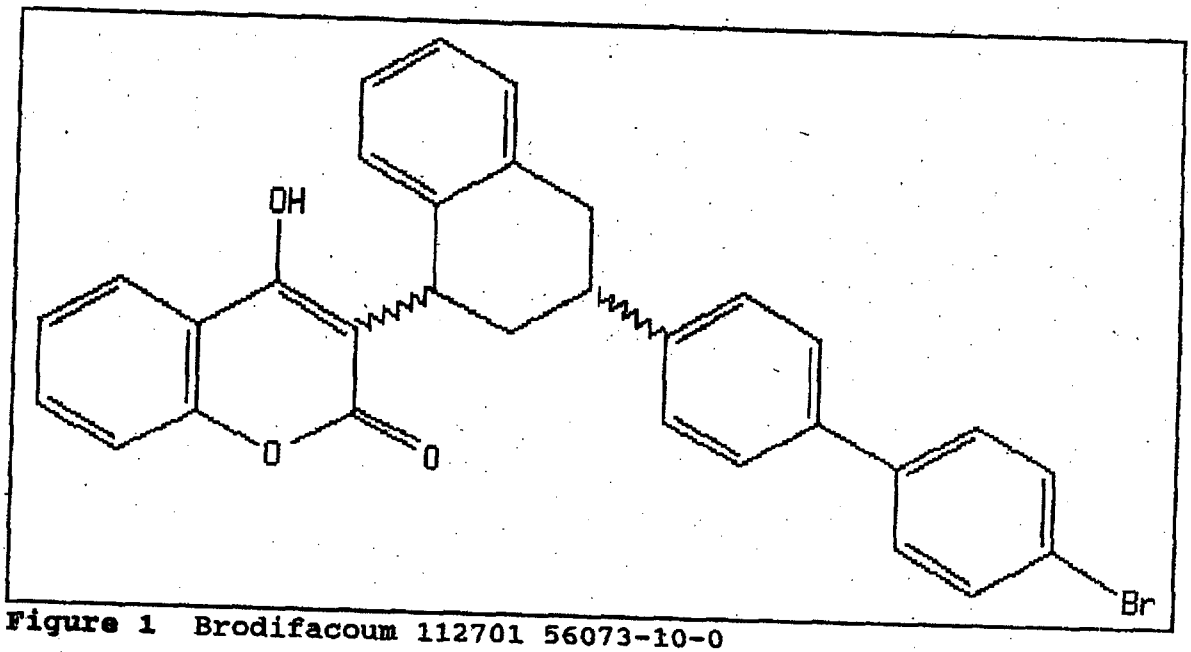

Figure 1 Brodifacoum 112701 56073-10-0

2. Vehicle: polyethylene glycol 300 (PEG 300)

3. Test animals: Species: rat
   Strain: Wistar-derived albino (Alpk:APfSD)
   Age: young adult
   Weight: males 224-311 g; females: 186-250 g.
   Source: Barriered Animal Breeding Unit, ZENECA Pharmaceuticals.
   Housing: 5 rats/cage, sexes kept separately
   Diet: Porton Combined Diet: ad libitum
   Water: [tap?] water ad libitum
   Environmental conditions:
   Temperature: 21 ± 2°C
   Relative humidity: 55 ± 15%

Air changes: 25-30/hour
Photoperiod: 12 hrs light/12 hrs dark
Acclimation period: "for a minimum of six days prior to the study."

B. STUDY DESIGN and METHODS:

1. In life dates - start: 22/09/92 (see p. 21) end: 8/11/92 (estimated from information p. 38)

2. Animal assignment and treatment - Animals were assigned to the test groups noted in table 1. Rats were fasted for a period of up to 24 hours, then were given a single dose of brodifacoum (96.1%) in PEG 300 by gavage then observed 1-2 times/day for 14 days. Rats were weighed on days -1, 1, 3, 5, 8 and 15 (day 1 was the day of dosing). Survivors were sacrificed and a necropsy was performed.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	males	Females	Combined
0.25	0/5	0/5	0/10
0.35	0/5	-	-
0.50	5/5	1/5	6/10
0.75	-	5/5	-

3. Statistics - From p. 12: "The acute oral median lethal dose was calculated from the mortality data (the mortality data included animals that were killed in extremis and/or showing signs of bleeding) by logistic regression using nominal dose values. Confidence limits for females were calculated using a likelihood ration interval... Approximate confidence limits for males are given by the highest dose with no mortalities and the lowest dose with 100% mortality. Slope estimates could not be calculated for either sex due to the mortality patterns."

II. **RESULTS AND DISCUSSION:**

A. Mortality is given in table 1. Deaths ranged from 3-8 days after administration (see p. 17 of the report).

The oral LD_{50} (C.I.) for males is 0.418 mg/kg (0.350-0.500 mg/kg)
for females is 0.561 mg/kg (0.472-0.667 mg/kg)
combined (not reported)

B. Clinical observations - There were no symptoms or mortalities at dose levels of 0.25 and 0.35 mg/kg. Signs of toxicity at higher dose levels included pallor and bruising and/or bleeding from the nose and/or rectum, and/or other sites. It is stated (p. 9) that: "signs of slight toxicity were observed in the surviving females [at 0.5 mg/kg];" however, the individual animal clinical observations for the 0.5 mg/kg females (refer to p. 22-25) indicate that one animal (#39) had piloerection on days 1-7, one (#40) had piloerection on day 1, and all had diarrhea on the day of dosing (presumably from the PEG 300).

C. Body Weight - From p. 13: "All animals lost weight initially due to the pre-dose fast but most had exceeded their day -1 bodyweight by day 3. Most of those animals surviving until termination continued to gain weight throughout the study. The majority of those animals which were found dead or were killed *in extremis* and/or because of bleeding showed a reduction in bodyweight prior to death."

D. Necropsy - From p. 14: "*Post mortem* examination of those animals which died or were killed *in extremis* and/or showing signs of bleeding revealed the presence of free or clotted blood in the abdominal and thoracic cavity... Discoloration or pallor of a variety of organs were also observed...

"In those animals surviving until termination, observations included red areas in the thymus, pallor of the kidney and renal pelvic dilatation." This probably refers to findings in two males (#432 and #435 - refer to p. 70 and 73) in the 0.35 mg/kg group.

E. Deficiencies - The study adequately defines the high toxicity of the test material (an oral LD_{50} of approximately 0.5 mg/kg, approximately 1% of the 50 mg/kg value which would place it in toxicity category I), and there are no substantive deficiencies.

012085

[BRODIFACOUM] Developmental Study OPPTS 870.3700 (§83-3(a))

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 6/10/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. C. Swentzel, Date 10/15/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - [rat]; OPPTS 870.3700 [§83-3 (a)]

DP BARCODE: D189478
P.C. CODE: 112701

SUBMISSION CODE: S437475
TOX. CHEM. NO.: 114AAA

TEST MATERIAL (PURITY): Brodifacoum (92.5%)

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

CITATIONS: Hodge, M.C.E.; Banham, P.B.; Richards, D.; et al. (1980) Brodifacoum: Teratogenicity Study in the Rat: Includes undated method entitled: The determination of Brodifacoum in dosing suspensions. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study No. RR0070, Report No. CTL/P/437, 22 January 1980. MRID 00052443. Unpublished.

Litchfield, M. (1980) Brodifacoum: Teratogenicity Study in the Rat: Individual Animal Data Supplement: Addendum to MRID #00052443. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID CTL/P/437S, 29 January 1980. MRID 40307202. Unpublished.

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EXECUTIVE SUMMARY: In a developmental toxicity study (MRIDs 00052443 and 40307202) brodifacoum (92.5%) was administered to 30 Alderley Park, Wistar-derived mated female rats/dose level by gavage in 10% v/v ethanol:water at dose levels of 0 (vehicle only), 0.001, 0.01 and 0.02 mg/kg/day from days 6 through 15 of gestation (as reported: in this study the morning on which spermatozoa were detected in vaginal smears was designated as day zero of gestation. In more recent studies the morning that sperm are detected is designated as day one of gestation).

There was blood in the uteri of one 0.01 and three

0.02 mg/kg females. This was considered to be possibly related to the administration of brodifacoum. There were no indications of any dose-related developmental effects associated with exposure to brodifacoum at doses up to and including 0.02 mg/kg/day. The dose level of 0.02 mg/kg/day is considered adequate, based on the occurrence of 100% mortality at a nominal value of 0.05 (analytical value of 0.35) mg/kg/day in a preliminary study, and blood measurements in a special study (Brodifacoum: Blood Kinetics Study in the Pregnant Rat, MRID 42641902).

The maternal NOEL is 0.001 mg brodifacoum/kg/day (based on the equivocal finding of blood in the uteri of one 0.01 and three 0.02 mg/kg females).

The developmental NOEL is 0.02 mg brodifacoum/kg/day (HDT).

This developmental toxicity study in the rat is classified as Acceptable (Guideline) (83-3a), and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat.

COMPLIANCE: A signed and dated Quality Assurance Statement is provided for the original study (p. 2 of MRID 00052443). Data Confidentiality, and Flagging statements are not provided in MRID 00052443. In MRID 92195013 [Titled: Phase Summary of MRIDs 52443 and 40307202; dated 24 April 1990] there is a signed and dated Statement of Data Confidentiality Claim (p. 2), and a Statement of Good Laboratory Practice Compliance (p. 3).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: brodifacoum, 92.5%
   Description: an off-white powder
   Batch #: 2,3,4,5 R1
   CTL Reference No.: Y00052/002/001
   Purity: 92.5%
   CAS No. 56073-10-0

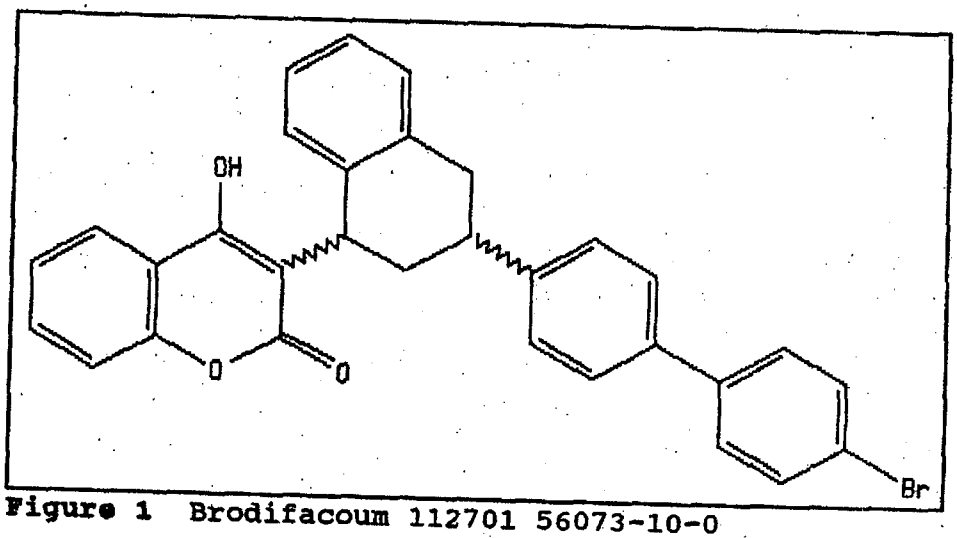

Figure 1 Brodifacoum 112701 56073-10-0

2. Vehicle: 10% v/v aqueous ethanol

3. Test animals: Species: rat
Strain: Alderley Park Wistar-derived (Alpk:SPfSD)
Age at mating: 13-14 wks (on arrival)
Weight at mating: 205 - 274g (on arrival)
Source: Colony maintained at Alderley Park; rats had already been mated before arrival. Supplied in four batches of animals (two batches of 28 and two of 32).
Housing: Individually in galvanized wire mesh cages in a Wilmslow-type mobile rat rack.
Diet: Pelleted Portion Combined Diet (PCD) supplied by BP Nutrition, Ltd. ad libitum
Water: tap water ad libitum
Environmental conditions:
Temperature: "19-23°C"
Relative humidity: usually 59-70%
Air changes: not stated
Photoperiod: 12 hrs light/ 12 hrs dark
Acclimation period: Not applicable because the females were mated at the breeding unit and then sent to the laboratory.

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 5 September, 1978 (animals were started on the study over a four-day period); end: September 23, 1978 [From p. 18 of MRID 00052443: period of dosing 11.9.78 to 23.9.78, with the footnote that: "since animals were started on the study over a four-day period, dosing was carried out over thirteen days."].

2. Mating: Virgin females were paired overnight with males of the same strain. On the following morning, vaginal smears from the females were examined for the presence of sperm. The day when sperm were detected was designated Day 0 of gestation and on this same day successfully mated females were delivered to the experimental unit at the Central Toxicology Laboratory.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was done according to a randomizing procedure; however, if several females had been mated with one male, these females "were distributed as evenly as possible between the groups to avoid bias due to that male."

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)[a]	Number of Females
Control	control (10% v/v aqueous ethanol	30
Low (LDT)	0.001	30
Mid (MDT)	0.01	30
High (HDT)	0.02	30

[a]Dosing was by gavage from days 6-15 (inclusive) with 1 ml dosing solution/100 g bodyweight.
Data Extracted from Table 1 of MRID 00052443 (p. 9).

4. Dose selection rationale: Dose levels were selected from the results of a preliminary teratogenicity range-finding study in the rat (mortalities summarized in Appendix 1 of MRID 00052443). In this preliminary study, all animals receiving doses ≥ 0.05 mg/kg/day died with thoracic hemorrhage.

5. Dosage preparation and analysis:

The report states that "the low solubility of brodifacoum in vehicles used normally for oral administration in teratogenicity experiments in this laboratory (corn or aqueous media) precluded the preparation of a true solution for dosing. It was also considered that because of the high toxicity of brodifacoum, the small amounts of compound needing to be mixed with a given volume of vehicle prevented the preparation of a suspension by conventional means. The solubility in

ethanol (0.1 g/100 ml) was sufficient to allow solution of the highest level of brodifacoum selected... It was anticipated that the addition of water to make a 10% aqueous solution would not cause brodifacoum to come out of solution. However, it was found that on standing, a colloidal suspension resulted."

"Samples were removed at intervals during the study for the analysis of brodifacoum content... Dosing suspensions containing 0.0001 mg/ml i.e. for the 0.001 mg/kg/day level were not analysed as the analytical method was not sufficiently sensitive to obtain quantitative values."

Results:

Homogeneity Analysis: In MRID 92195013 it is stated (p. 6) that: "Homogeneity of the dosing suspensions was not assessed. However, a colloidal suspension was formed and it is reasonable to assume that the homogeneity would be acceptable."

Stability Analysis: See Table 2 (below).

Concentration Analysis: The analyzed concentrations of brodifacoum in suspensions ranged from 30 to 110% of nominal values; however, the range was somewhat

narrower (60-105%) for the highest dose (0.02 mg/kg).

TABLE 2 Stability and Concentration Analyses

Sample/Dose Level (mg/kg/day)	Date of Preparation	Date of Analysis	Dates Used	Brodifacoum Content (mg/ml) Theoretical	Brodifacoum Content (mg/ml) Found
Stock Suspension	30.8.78	7.9.78 12.9.78	11.9.78 to 19.9.78	0.015	0.013 0.013
Stock Suspension	14.9.78	15.9.78 21.9.78 22.9.78	20.9.78 to 23.9.78	0.015	0.015 0.014 0.015
0.01 0.02	11.9.78	11.9.78	11.9.78	0.001 0.002	0.0007 0.0017
0.01 0.02	12.9.78	12.9.78	12.9.78	0.001 0.002	0.0010 0.0020
0.01 0.02	15.9.78	15.9.78	15.9.78	0.001 0.002	0.0011 0.0021
0.01 0.02	20.9.78	21.9.78	20.9.78	0.001 0.002	0.0005 0.0014
0.01 0.02	21.9.78	21.9.78	21.9.78	0.001 0.002	0.0003 0.0012
0.01 0.02	22.9.78	22.9.78	22.9.78	0.001 0.002	0.0005 0.0016

Data extracted from Table 2 of MRID 00052443 (p. 18).

It is stated (p. 28 of MRID 00052443) for the preliminary range-finding study in the rat that: "...the brodifacoum analysis of the first two sets of suspensions was below the theoretical levels. This was explained when it was found that if the dosing suspensions were stored in plastic bottles the concentrations were reduced, presumably because the compound was adsorbed onto the plastic. The stock suspensions, however, were kept in glass bottles and did not show the same...variation. The last set of suspensions was, therefore, kept in glass bottles and the actual concentrations were within 10% of nominal. All levels referred to in the results are the nominal ones. No satisfactory explanation has been found for the positive analysis in the group 1 control sample since the concentration found would require a high level of contamination." [Note by reviewer: this last sentence is difficult to interpret, as - refer to page 31 of MRID 00052443 - brodifacoum analyses results for control samples ranged from not detected to 0.0004 mg/ml; in the analyses done June 30, 1978 the respective values for nominal value solutions of

0, 0.001, 0.002 and 0.015 mg/ml are reported as: nd (=none detected), 0.0095, 0.0022 and 0.015 mg/ml; it is presumed that the 0.0095 value is a typographical error for 0.00095.]

6. Dosage administration: All doses were administered once daily by gavage on gestation days 6 through 15 (inclusive) in a volume of 10 ml/kg body weight/day. It is noted (see p. 10 of MRID 00052443) that: "dosing suspensions were prepared daily by diluting aliquots of the stock suspension with 10% v/v aqueous ethanol."

C. OBSERVATIONS

1. Maternal Observations and Evaluations: Dams were checked daily for mortality or clinical signs. Body weights were recorded on days 0, 6 through 15 and 21 of gestation. There are no individual or mean group food consumption data in either MRID 00052443 or 40307202. Dams were sacrificed on day 21 of gestation. At sacrifice, "an autopsy was carried out and the tissues of all females were examined macroscopically. The liver and kidneys from twenty pregnant females per groups were submitted in formol corrosive for histopathological examination, together with abnormal tissues from any animal examined." A number of organs were stored in formol saline, and 5 µm sections were prepared and stained. "Only tissues from animals receiving 0.02 mg/kg/day and controls were examined."

2. Fetal Evaluations: During the autopsy, the intact gravid uterus was removed and weighed. The following data were recorded: number of live fetuses and resorptions (the latter classified as early or late), and number of corpora lutea. "The weight and external sex of each individual foetus were recorded, and at the same time viability was assessed using the following criteria - colour, breathing and movement. Any reduction in viability was noted. "The fetuses were weighed, then fetuses from all litters "were examined for malformations including cleft palate and any abnormality was recorded, although only those offspring of females from which tissues were taken for pathological examination (approximately twenty per group) were processed for examination of either the skeleton or the soft tissues." In MRID 92195013 it is stated (p. 6) that: "Only twenty litters per group were examined, any additional litters were discarded unless abnormalities were seen."

"For skeletal examination, alternate foetuses (starting randomly within each litter) were fixed in 70% methanol. Approximately 24 hours later they were eviscerated (the viscera being examined macroscopically for abnormalities) and the fat pads covering the cervical and thoracic vertebrae were removed. The internal sex was checked against that recorded externally. Each foetus was...processed and stained with Alizarin Red S using the method of Staples and Schnell...for subsequent skeletal examination... ossified bones were examined both for abnormalities and for degree of ossification."

The overall ossification of forelimb and hindlimb digits was assessed on a four point scale:

Scale for assessment of skeletal ossification of digits (from Appendix 6, p. 38):

1. (good) Metacarpals/metatarsals and first and third rows of phalanges fully ossified (or one phalanx partially ossified).

2. Metacarpals/metatarsals fully ossified. 1st and 3rd rows of phalanges fully ossified, although an occasional phalanx (up to 4) may be partially ossified.

3. Metacarpals/metatarsals fully or occasionally partially ossified. First row of phalanges either partially or not ossified together with third row of phalanges either partially or fully ossified.

4. (poor) Metacarpals/metatarsals - some either partially ossified or not ossified plus first row of phalanges usually not ossified and third row of phalanges partially ossified.

D. DATA ANALYSIS:

1. Statistical analyses: From p. 12: "Statistical analysis was carried out by comparing results from each brodifacoum group with results from the control group. The following mean values were analysed by Student's t-test: maternal weight gain, gravid uterus weight, litter weight (calculated from the total weight of live foetuses per litter), foetal weight (calculated from values for the mean per litter), number of implantation sites, viable foetuses and corpora lutea. Pre- and post-

implantation losses were similarly analyzed..."

"The percentages of early and late resorptions and of male foetuses were also calculated for each group."

"Skeletal and soft tissue findings in the foetuses were analysed by the use of 2 x 2 contingency tables... This test is useful for an initial examination of such results although possible effects many need to be investigated by more sophisticated methods."

2. Indices: The following indices were calculated from cesarean section records of animals in the study:

$$\% \text{ pre-implantation loss} = \frac{\#\text{ corpora lutea} - \#\text{ implantations}}{\#\text{ corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\#\text{ implantations} - \#\text{ live fetuses}}{\#\text{ implantations}} \times 100$$

3. Historical control data: No historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: From p. 12: "No clinical findings were recorded which could be attributed to the administration of brodifacoum. Female 94 receiving 0.02 mg/kg lacked upper incisors and was killed on Day 6. Female 53 receiving 0.001 mg/kg littered on Day 21 and was excluded from the pregnancy and litter results. Female 1 (control) was recorded to have rapid respiration and dark eyes on Day 10 only."

2. Body Weight: The statement is made (p. 14): that: "Mean bodyweight and bodyweight gain from Days 0-21 were similar for females of all groups."

TABLE 3 Maternal Body Weight Gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (25)	0.001 (26)	0.01 (27)	0.02 (24)
Pretreatment: Days 1 - 6	33.5	30.3	31.4	31.5
Treatment: Days 6 - 15	46.4	45.9	47.6	48.5
Posttreatment: Days 15-21	77.6	81.7	81.5	81.3
Overall: Days 1 - 21	157.5	157.9	160.5	161.3

a Data calculated from information in Table 3 of report CTL/P/437 (p. 19 of MRID 00052443) and pages 6-9 of MRID 40307202.

3. Gross Pathology: From p. 14: "The uteri of three females receiving 0.02 mg/kg and one female receiving 0.01 mg/kg contained blood. Macroscopic examination of female 101 receiving 0.02 mg/kg revealed that the bladder contained red fluid and the kidneys were pale with slight pelvic dilatation. These findings were interpreted microscopically as a severe haemorrhagic cystitis and ascending pyelonephritis."

"The lungs of several females from all groups were noted to be patchy and not deflated at autopsy. The animals [lungs?] examined microscopically showed haemorrhages. These findings were considered to be the result of killing by cervical dislocation."

"None of the findings with the possible exception of the presence of blood in the uterus were considered to be related to the administration of brodifacoum."

4. Cesarean Section Data: No treatment-related effects were observed. From p. 14 of MRID 00052443: "The administration of brodifacoum had no adverse effect on any of the parameters monitored." The following table summarizes the results.

TABLE 4 Cesarean Section Observations[a]

Observation	Dose (mg/kg/day)			
	0	LDT (0.001)	MDT (0.01)	HDT (0.02)
# Animals Assigned (Mated)	30	30	30	30
# Animals Pregnant Pregnancy Rate (%)	25 (83)	26 (87)	27 (90)	24 (80)
Total # Corpora Lutea* Corpora Lutea/Dam (±S.D.)	323* 13.46 (±1.38)	323* 12.92 (±1.79)	334* 12.85 (±1.26)	298* 12.96 (±1.52)
Total # Implantations Implantations/Dam	308 12.3	317 12.2	332 12.3	296 12.3
Total # Litters	25	26	27	24
Total # Live Fetuses Live Fetuses/Dam	290 11.6	311 12.0	321 11.9	288 12.0
Total # Dead Fetuses Dead Fetuses/Dam	0 0	0 0	0 0	0 0
Total # Resorptions Early Late Resorptions/Dam Early Late Litters with Total Resorptions	18 8 10 0.72 0.32 0.4 0	6 5 1 0.23 0.19 0.04 0	11 10 1 0.41 0.37 0.04 0	8 7 1 0.33 0.29 0.04 0
Mean Gravid Uterus Weight (g)	82.2[b]	82.7[c]	83.6[c]	85.2
Mean Litter Weight Mean Fetal Weight (g)[d]	58.8 5.07	61.1 5.11	62.2 5.23	62.7 5.23
Sex Ratio (% Male)	50.0	52.1	50.8	50.0
Preimplantation Loss (%)	8.6	6.6	5.8	6.8
Postimplantation Loss (%)	5.8 3.0[e]	2.6	3.3	2.7

* Number of corpora lutea not recorded from one dam in each dose group.

a Data extracted (and calculated) from Table 5a of MRID 00052443
and Appendix C (pages 49-82 and 84-87) of MRID 40307202.

b Excludes uterus weight from one female with 9 late resorptions and only 3 live fetuses; with this uterus the mean weight is 80.4 g.

c Gravid uterus of one animal was not weighed

d Total fetal weight ÷ number of fetuses

e Excluding results from female with 9 late resorptions and only 3 live fetuses

B. DEVELOPMENTAL TOXICITY

No treatment-related differences between the treated and control groups were observed for either the external/visceral or the skeletal examinations. There were statistically significant differences for specific observations, however, there was no dose-response.

TABLE 5 Intergroup Comparison of Soft Tissue Abnormalities[a]

Observation	Dose Level of Brodifacoum (mg/kg/day)			
	0	0.001	0.01	0.02
Number of fetuses examined	115	121	125	112
Kidneys				
Right: slight pelvic dilatation	5	6	9	7
Left: slight pelvic dilatation	5	4	8	6
Both: slight pelvic dilatation	3	3	2	2
Right: moderate pelvic dilatation	0	0	1	0
Number of fetuses affected	13	13	20	15
Number of litters affected	9/25	8/26	13/27	12/24
Ureter				
Right: dilated with constrictions	0	0	1	0
Brain				
Hydrocephalus: mild	1	0	0	0
Hydrocephalus: severe	1	0	0	1
Head				
Agnathia: no tongue or buccal cavity. One nasal passage.	0	0	1	0

[a]Extracted from Table 6, p. 23 of MRID 00052443

TABLE 6 Skeletal Examination

Dose Levels (mg/kg/day)	0		0.001		0.01		0.02	
Observation	No.	%	No.	%	No.	%	No.	%
Number of fetuses examined	114		121		126		118	
Skull								
Slightly widened fontanelle	6	5.3	9	7.4	7	5.6	1	0.8
Moderately widened fontanelle	1	0.9	0	0.0	0	0.0	0	0.0
Nasals partially ossified	0	0.0	1	0.8	0	0.0	0	0.0
Frontals partially ossified	0	0.0	3	2.5	0	0.0	0	0.0
Parietals partially ossified	3	2.6	2	1.7	0	0.0	0	0.0
Interparietals partially ossified	1	0.9	3	2.5	2	1.6	2	1.7
Occipitals partially ossified	0	0.0	1	0.8	0	0.0	0	0.0
Vertebrae								
Cervical								
Centra 1-2 not ossified	5	4.4	10	8.3	3	2.4	3	2.5
Centra 1-3 not ossified	1	0.9	4	3.3	0	0.0	0	0.0
Centra 1-4 not ossified	2	1.8	0	0.0	0	0.0	0	0.0
Centra 1-5 not ossified	1	0.9	0	0.0	3	2.4	0	0.0
Centra 1-6 not ossified	4	3.5	1	0.8	0	0.0	0	0.0
Centra 1-7 not ossified	0	0.0	1	0.8	0	0.0	0	0.0
Centra 2-3 not ossified	1	0.9	1	0.8	1	0.8	3	2.5
Centra 2-4 not ossified	0	0.0	0	0.0	1	0.8	0	0.0
Centra 2-5 not ossified	1	0.9	1	0.8	0	0.0	0	0.0
Centra 4-5 not ossified	0	0.0	1	0.8	0	0.0	0	0.0
Centrum 1 not ossified	13	11.4	9	7.4	5	4.0	6	5.1
Centrum 2 not ossified	20	17.5	24	19.8	16	12.7	18	15.3
Centrum 3 not ossified	2	1.8	1	0.8	2	1.6	0	0.0
Centrum 4 not ossified	0	0.0	0	0.0	1	0.8	0	0.0
Thoracic								
Centra 9-13 partially ossified	1	0.9	0	0.0	0	0.0	0	0.0
Centra 10-13 partially ossified	1	0.9	0	0.0	0	0.0	0	0.0
Lumbar								
Centra 1-3 partially ossified	1	0.9	0	0.0	0	0.0	0	0.0
Centrum 1 partially ossified	1	0.9	0	0.0	0	0.0	0	0.0
Total number of vertebrae ossified								
33 ossified	1	0.9	1	0.8	0	0.0	0	0.0
34 ossified	4	3.5	8	6.6	4	3.2	2	1.7
35 ossified	24	21.1	23	19.0	24	19.0	20	16.9
36 ossified	33	28.9	35	28.9	38	30.2	33	28.0
37 ossified	33	28.9	36	29.8	37	29.4	33	28.0
38 ossified	15	13.2	14	11.6	15	11.9	22	18.6
39 ossified	4	3.5	3	2.5	7	5.6	6	5.1
40 ossified	0	0.0	1	0.8	1	0.8	2	1.7

Extracted from Table 7, p. 24-25 of MRID 00052443

TABLE 6 Skeletal Examination (continued)

Dose Levels (mg/kg/day)	0		0.001		0.01		0.02	
Observation	No.	%	No.	%	No.	%	No.	%
Number of fetuses examined	114		121		126		118	
Ribs								
14 left - short	6	5.3	3	2.5	8	6.3	4	3.4
14 right - short	3	2.6	5	4.1	3	2.4	4	3.4
14 bilateral - short	12	10.5	15	12.4	9	7.1	12	10.2
14 bilateral - 1 long, 1 short	1	0.9	0	0.0	1	0.8	0	0.0
Number of animals with extra ribs	22	19.3	23	19.0	21	16.7	20	16.9
Sternebrae								
Total not ossified	2	1.8	1	0.8	3	2.4	0	0.0
Total partially ossified	100	87.7	94*	77.7	86**	68.3	71**	60.2
Total abnormal	26	22.8	15*	12.4	6**	48.0	5**	4.2
Forelimb								
Assessment of ossification of digits:								
Grade 1 (good)	14	12.3	20	16.5	22	17.5	35**	29.7
Grade 2	62	54.4	67	55.4	72	57.1	61	51.7
Grade 3	31	27.2	32	26.4	29	23.0	21	17.8
Grade 4 (poor)	7	6.1	2	1.7	3	2.4	1	0.8
Hindlimb								
Assessment of ossification of digits								
Grade 1 (good)	15	13.2	8	6.6	14	11.1	19	16.1
Grade 2	37	32.5	40	33.1	58*	46.0	52	44.1
Grade 3	39	34.2	42	34.7	34	27.0	34	28.8
Grade 4 (poor)	23	20.2	31	25.6	20	15.9	13	11.0
Calcaneum fully ossified	2	1.8	2	1.7	5	4.0	3	2.5
Calcaneum partially ossified	22	19.3	16	13.2	46**	36.5	39*	33.1

* = Significantly different from control fetal incidence: $p \leq 0.05$
** = Significantly different from control fetal incidence: $p \leq 0.01$
Extracted from Table 7, p. 25-26 of MRID 00052443

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: There was no indication of an increased incidence of any fetal soft tissue and/or skeletal abnormality associated with maternal exposure to brodifacoum.

It is emphasized by the investigators (see p. 15 of MRID 00052443) that:

"Prior to the start of the experiment, the greatest difficulty was in selecting suitable dose-levels. As might be expected from a chemical intended for use as a rodenticide, brodifacoum is an extremely toxic material to the rat. In the range-finding studies...a theoretical level of 0.05 mg/kg (analytical level of 0.035 mg/kg) was found to be lethal to pregnant rats after several consecutive daily doses, while 0.02 mg/kg did not cause any observable effect. It would appear that with this particular anti-coagulant there is a very steep dose-response curve, at least with pregnant rats, which gives an apparent 'all or none' response even when blood coagulation times were measured as they were in the preliminary study [note by reviewer: blood coagulation times for the preliminary study are not reported in either MRID 00052443 or 40307202]... In view of the sharp cut-off point with brodifacoum between the measured levels of 0.02 and 0.035 mg/kg, 0.02 mg/kg was considered to be the maximum dose-level which could be used for the teratogenicity experiment since a higher level was likely to produce an unacceptable mortality rate. The presence of blood in three gravid uteri from animals which had received 0.02 mg/kg and in the bladder of one non-pregnant female could have been evidence of an anti-coagulant effect, although this is by no means conclusive since such observations are occasionally made in control rats."

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: There was no indication of any effects on mean maternal body weights, or any other observational parameter. The only possible effect was the presence of blood in the gravid uteri of three rabbits which received 0.02 mg/kg/day, and one female which received 0.01 mg/kg/day. In addition, female 101 (dosed at 0.02 mg/kg/day, but non-pregnant) is reported (see p. 39 of MRID 40307202) as "losing dark red fluid from vulva," and having submucosal hemorrhages in the bladder.

2. DEVELOPMENTAL TOXICITY: There were no indications of developmental toxicity in this study. Therefore, the NOEL for developmental toxicity for brodifacoum is 0.02 mg/kg/day.

C. STUDY DEFICIENCIES AND ITS ADEQUACY: This study was previously reviewed by Dr. Zendzian (Oct. 26, 1989; Caswell file document 007588). In that review, it was stated: "The reports remain unacceptable. Individual observations on the dams are missing from the supplement to the report of the rat study and individual observations on the fetal skeletons are missing from both supplements."

"Based on the data provided the studies will be, at best, supplementary. Due to the cumulative toxicity of brodifacoum, maximum fetal exposure was not achieved until the end of and after the critical period of fetal development."

It is noted that MRID 40307202 includes (pages 11-47, pages 94-96) individual pathology data for 20 control and 19 highest-dose (0.02 mg/kg/day) dams; three of the latter (#97; refer to p. 36; #105, refer to p. 42; and #110; refer to p. 95) are reported as having blood in the uterus (in each case this finding was macroscopically evident).

Although no individual observations on fetal skeletons are provided, the fetal incidence findings (refer to Table 7 in MRID 00052443, duplicated in Table 6 of this DER) adequately demonstrate a lack of developmental toxicity involving skeletal development.

The registrant has provided data (a study titled: Brodifacoum: Blood Kinetics Study in the Pregnant Rat, MRID 42641902) showing that the concentration of brodifacoum in maternal blood of rats receiving 0.02 mg/kg/day from days 7-16 is, during the period from days 11-15, between 2 and 3 ng equivalents brodifacoum/g of blood, essentially the same during this period as for rats receiving 0.0125 mg/kg from days 1-16. Presumably then, rats which received 0.02 mg/kg/day in the period from days 6-15 of gestation would have had blood levels of between 2 and 3 ng equivalents brodifacoum/g of blood during the period from days 10-14.

It is concluded that the report, in conjunction with the material in MRID 42641902, provides sufficient information to adequately define the maternal and fetal toxicity potential of brodifacoum.

012085

[BRODIFACOUM]

Developmental Study OPPTS (§83-3(b))

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 10/11/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. C. Swentzel, Date 10/15/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - [rabbit]; OPPTS 870.3700 [§83-3 (b)]

DP BARCODE: D189478
P.C. CODE: 112701

SUBMISSION CODE: S437475
TOX. CHEM. NO.: 114AAA

TEST MATERIAL (PURITY): Brodifacoum (92.5%)

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

CITATIONS: Hodge, M.C.E.; Banham, P.B.; Richards, D.; et al. (1980) Brodifacoum: Teratogenicity Study in the Rabbit: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study No. RB0071, Report No. CTL/P/459, 30 January 1980. MRID 00052442. Unpublished.

Litchfield, M. (1980) Brodifacoum: Teratogenicity Study in the Rabbit: Individual Animal Data Supplement: Addendum to MRID #00052442. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID CTL/P/459S, 29 January 1980. MRID 40307201. Unpublished.

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EXECUTIVE SUMMARY: In a developmental toxicity study (MRIDs 00052442 and 40307201) brodifacoum (92.5%) was administered to 15 mated female Dutch rabbits/dose level by gavage in 5% v/v ethanol:water at dose levels of 0 (0.5% v/v aqueous Tween 80), 0 (5% v/v aqueous ethanol, the vehicle used with brodifacoum), 0.001, 0.002 and 0.005 mg brodifacoum/kg/day from days 6 through 18 of gestation (in this study gestation day 0 was the day of mating. In more recent studies it is designated as day one of gestation).

Ten of the 15 rabbits receiving 0.005 mg/kg/day died or were humanely killed; all were found to have internal hemorrhage. Nine of these does had loss of blood (in some cases heavy) from the vagina. All of

the implants of one doe (#47; killed on day 16) in the 0.005 mg/kg/day group are reported to have had a hemorrhagic appearance, but otherwise there were no indications of any dose-related developmental or toxic effects associated with exposure to brodifacoum at doses up to and including 0.005 mg/kg/day. Because only three litters (and only 20 fetuses) were available from the 0.005 mg/kg/day group at 29 days (and taking into consideration the hemorrhagic appearance of the implants of #46), the NOEL for fetal toxicity is 0.002 mg/kg/day, and the LOEL is 0.005 mg/kg/day. The only possible indication of toxicity in the 0.002 mg/kg/day does was the occurrence of a small hemorrhage beneath the lid of one eye on gestation day 14 in one rabbit (#44) which was not pregnant, but a similar finding is not reported for the 0.005 mg/kg/day females.

The maternal NOEL is 0.002 mg brodifacoum/kg/day. The LOEL is 0.005 mg/kg/day (based on 75% mortality associated with hemorrhage in pregnant females at this dose level). In addition, the prothrombin time was significantly increased at 0.005 mg/kg/day on day 20 relative to controls (to 26.5 [seconds?] from 14.5) in a preliminary range-finding study. The developmental toxicity NOEL is 0.002 mg/kg/day, as only 3 litters (with a total of 20 fetuses) were available at 0.005 mg/kg/day), and it is reported that all the implants from a 0.005 mg/kg/day doe which was killed on day 16 had a hemorrhagic appearance.

This developmental toxicity study in the rabbit is classified as Acceptable (Guideline) (83-3b).

COMPLIANCE: A signed and dated Quality Assurance Statement is provided for the original study (p. 2 of MRID 00052442). Data Confidentiality, and Flagging statements are not provided in MRID 00052442. In MRID 92195015 [Titled: Phase 3 Summary of MRIDs 52442 and 40307201; dated 25 April 1990] there is a signed and dated Statement of Data Confidentiality Claim (p. 2), and a Statement of Good Laboratory Practice Compliance (p. 3).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: brodifacoum, 92.5%
   Description: an off-white powder
   Batch #: 2,3,4,5 R1
   CTL Reference No.: Y00052/002/001
   Purity: 92.5%
   CAS No. 56073-10-0

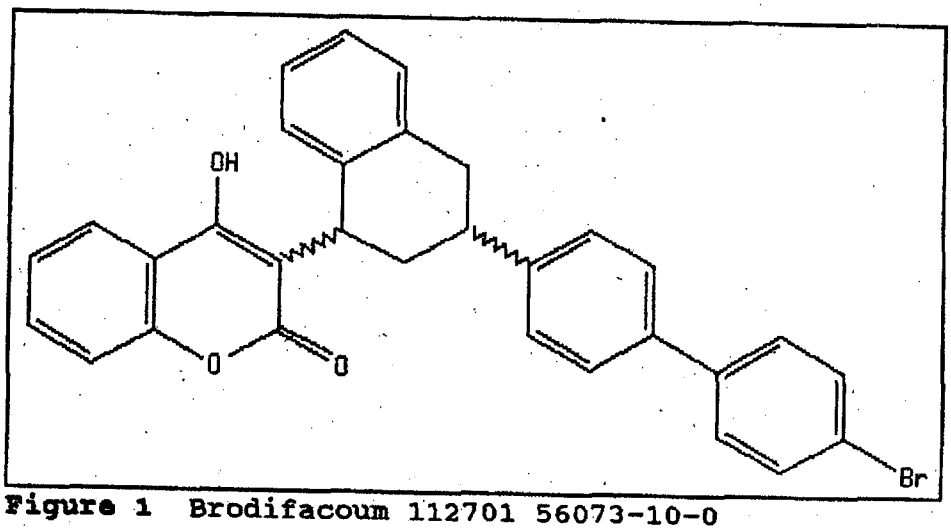

Figure 1 Brodifacoum 112701 56073-10-0

2. Vehicle: 5% v/v aqueous ethanol

3. Test animals: Species: rabbit
Strain: Dutch
Age at mating: not given
Weight at mating: 1.65-3.15
Source: Ranch Rabbits, Crawley Down, Sussex, UK
Housing: Individually in metal cages
Diet: CRB rabbit pellets ad libitum
Water: tap water ad libitum
Environmental conditions:
Temperature: "13-22°C"
Relative humidity: 55-65%
Air changes: not stated
Photoperiod: 12 hrs light/12 hrs dark
Acclimation period: Ranging from 2 to 21 weeks

B. PROCEDURES AND STUDY DESIGN

1. In life dates - The period of dosing is reported (p. 18) as being from 7 Jan. 1979 to 29 Jan. 1979, with the note that "Since animals were started on the study over an eleven-day period, dosing was carried out over 23 days." Since does were dosed on days 6-18 of pregnancy, it can be calculated that the period of pregnancy was from 1 Jan. 1979 to 9 Feb. 1979. This agrees with the statement (p. 7) that: "The study began (Day 0 of pregnancy) on 1 January 1979 and finished on 9 February 1979."

2. Mating: From p. 9: "Does were mated with the untreated bucks... Each doe was placed in a buck's cage... Coitus was observed and, if possible, two matings (with the same buck) were allowed to take place... The fertility of each buck was confirmed by the presence of live sperm in the ejaculate as shown by the vaginal smear examination of a recently-mated doe. Approximately one hour after mating, each doe was given an intravenous injection of chorionic gonadotrophin...in order to promote ovulation. The day of mating was considered to be Day 0 of pregnancy."

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. From p. 9: "On Day 0 of pregnancy mated does were randomly allocated to five experimental groups..." From p. 34: "the females mated with any given male were distributed evenly among groups. This was done to reduce any possible bias due to the males..."

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)[a]	Number of Females
Control #1	0.5% v/v Tween 80	15
Control #2	vehicle (5% v/v aqueous ethanol	15
Low (LDT)	0.001	15
Mid (MDT)	0.002	15
High (HDT)	0.005	15

[a]Dosing was by gavage from days 6-18 (inclusive) with 2 ml dosing solution/kg bodyweight.
Data Extracted from Table 1 of MRID 00052442 (p. 10).

4. Dose selection rationale: Dose levels were selected from the results of a preliminary range-finding study (summarized in Appendix 1 - pp. 27-31 - of MRID 00052442). In this preliminary study, all animals receiving doses > 0.005 mg/kg/day, as well as 2/5 at 0.005 mg/kg/day, died with thoracic and/or uterine hemorrhage. In this preliminary range-finding study, 4 control animals, two receiving 0.001 and three receiving 0.005 mg/kg/day were killed on Day 20, and blood samples were taken for clotting function. Results are shown in Table 2:

Table 2. Prothrombin Time in the Preliminary Developmental Toxicity Range-Finding Study in the Rabbit (Day 20)

	Control	0.001 mg/kg/day	0.005 mg/kg/day
Mean	14.5	17.4	26.5**
SD	2.0	-	5.1
No. of samples	4	1	3

** Statistically significant at the 1% level (Student's t-test) compared with the control group

Data Extracted from appendix 1 of MRID 00052442 (p. 31).

5. Dosage preparation and analysis:
The report states (p. 10) that: "the low solubility of brodifacoum in the aqueous media normally used as vehicles for oral administration precluded the preparation of a true solution for dosing. It was also considered that the small amounts of compound needing to be mixed with a given volume of vehicle because of the high toxicity of brodifacoum prevented the preparation of a suspension by conventional means. The solubility in ethanol (0.1 g/100 ml) was sufficient to allow solution of the highest level of brodifacoum selected for the experiment. It was expected that the addition of water to make a 10% v/v aqueous ethanol solution [note by reviewer: the 10% v/v ethanol solution was used in the practically concurrent rat developmental study; a 5% v/v solution was used in this rabbit study] would not cause brodifacoum to come out of solution. It was found, however, that on standing a colloidal suspension resulted."

"Dosing suspensions were prepared from a stock suspension containing a theoretical concentration of 0.05 mg/ml which was made up by dissolving the required amount of test material in ethanol and diluting to volume with water to give a final ethanol concentration of 5% v/v. The dosing suspensions were prepared daily by diluting aliquots of the stock suspension with 5% v/v aqueous ethanol..."

"Samples were taken at intervals during the study and [were] analysed for brodifacoum using...two [analytical] methods..."

Results: Homogeneity Analysis: In MRID 92195015 it is stated (p. 5) that: "Homogeneity of the dosing suspensions was not assessed. However, a colloidal suspension was formed and it is reasonable to assume that the homogeneity would be acceptable."

Stability Analysis: See Table 3 (below).

Concentration Analysis: From p. 14: "Analysis of the dosing suspensions showed that the brodifacoum content was 76-90% of the expected nominal content in the three dose groups. Refer to Table 3 (below):

Table 3. Analysis of stock suspensions and dosing solutions for brodifacoum content

Sample (mg/ml) Brodifacoum	Date of Preparation	Date of Analysis	Method of Analysis	Brodifacoum Content Found (mg/ml)	Mean (mg/ml)*
Stock Suspension 0.05	18.12.78	21.12.78	A	0.055	0.055
Control (5% v/v ethanol)	18.12.78	9.1.79	A	0	0
		6.2.79	B	Trace	<0.00002
0.0005	4.1.79	9.1.79 12.1.79 19.1.79	A	0.0003 0.0004 0.0006	0.0004
		6.2.79	B	0.0004	0.0004
0.001	4.1.79	9.1.79 12.1.79 19.1.79	A	0.0010 0.0007 0.0011	0.0009
		6.2.79	B	0.0008	0.0008
0.0025	4.1.79	9.1.79 12.1.79 19.1.79	A	0.0023 0.0020 0.0016	0.0020
		6.2.79	B	0.0019	0.0019

Data extracted from Table 2 of MRID 00052442 (p. 18).

Method of analysis A: Portions of each sample were injected directly onto a reversed-phase liquid chromatography column and brodifacoum eluted using ethanol as the mobile phase. Brodifacoum was detected by passing the column eluent through a fluorescence detector, with calibration using a diluted standard solution.

Method of analysis B: Brodifacoum suspended in 5% v/v aqueous ethanol was extracted with ethyl acetate. The extract was evaporated to dryness, the brodifacoum residue was dissolved in ethanol and then was analysed by liquid chromatography with a fluorescence detector (as in Method A).

It is noted (p. 28 of MRID 00052443) for the preliminary range-finding study in the rat that: "...the brodifacoum analysis of the first two sets of suspensions was below the theoretical levels. This was explained when it was found that if the dosing suspensions were stored in plastic bottles the concentrations were reduced, presumably because the compound was adsorbed onto the plastic. The stock suspensions, however, were kept in glass bottles and did not show the same...variation." The dates for analysis of stock and dosing solutions for the rat study were from August 30, 1978 through September 22, 1978, and for the rabbit study were from December 21, 1978 through January 19, 1979, so that presumably the dosing solutions in the (subsequent) rabbit study were in kept in glass (rather than plastic) bottles,

6. Dosage administration: All doses were administered once daily by gavage on gestation days 6 through 15 (inclusive) in a volume of 10 ml/kg body weight/day. It is noted (see p. 10 of MRID 00052443) that: "dosing suspensions were prepared daily by diluting aliquots of the stock suspension with 10% v/v aqueous ethanol."

C. OBSERVATIONS

1. Maternal Observations and Evaluations: From p. 11: "Prior to mating, the health status of each animal was checked and only healthy animals were mated. The clinical condition of all animals was monitored daily during the experiment and any abnormalities recorded... On day 29 of pregnancy the does were killed by air embolism. An autopsy was carried out and the tissues of all animals were examined macroscopically." Some organs were stored in formol saline, but were not further processed or examined histopathologically.

2. Fetal Evaluations: During the autopsy, the intact gravid uterus was examined for number of live fetuses and resorptions (classified as early or late). Numbers of corpora lutea were also determined. In utero positions of fetuses were recorded, individual weights were determined, and viability was assessed. The fetuses were sexed on later internal examination.

"Foetuses from all litters were examined externally for malformations, including cleft palate, and any abnormalities were recorded. The foetuses were then processed for examination of either the skeleton or the soft tissues. For skeletal examination: alternate foetuses (starting randomly within each litter) were fixed in 70% methanol. Approximately 24 hours later they were eviscerated (the viscera being examined by naked eye for abnormalities), skinned and the fat pads covering the cervical and thoracic vertebrae removed. Each foetus was returned to methanol, then processed and stained with Alizarin Red S using the method of Staples and Schnell...for subsequent skeletal examination... ossified bones were examined for abnormalities and degree of ossification. The ossification of forelimb and hindlimb digits was assessed on a four-point scale...although all individual bones were examined."

The overall ossification of forelimb and hindlimb digits was assessed on a "four-point scale" (this is what it says on page 12, although what is given in appendix 6 is a five-point scale):

<u>Scale for assessment of skeletal ossification of digits</u> (from Appendix 6, p. 40):

1. (good) Metacarpals/metatarsals and 1st, 2nd and 3rd rows of phalanges fully ossified.

2. Metacarpals/metatarsals and 1st and 3rd rows of phalanges fully ossified, some of 2nd row not ossified.

3. Metacarpals/metatarsals fully ossified. All 1st and 3rd row present, the majority being fully ossified, most of 2nd row not ossified although occasional phalanx may be partially ossified.

4. One metacarpal or metatarsal may be partially ossified, remainder of the metatarsals or metacarpals fully ossified. 2nd row of phalanges not ossified, most of 1st and 3rd rows of phalanges fully ossified but a few partially ossified.

5. One metacarpal or metatarsal partially ossified or not ossified, remainder of metatarsals and metacarpals fully ossified. 2nd row of phalanges not ossified, occasional phalanges in 1st and 3rd row not ossified, remainder partially ossified.

"The remaining foetuses were preserved and decalcified in Bouin's fixative for at least eight weeks prior to examination. Sections were made through the head according to the technique of Wilson...while the thorax and abdomen were dissected, sections being made through the heart, lungs, liver and kidneys in order to examined their internal structure. Each person engaged in foetal examination examined a similar proportion of litters from each group in order to avoid operator bias."

D. DATA ANALYSIS

1. Statistical analyses: From p. 13: "The following mean values were analysed by Student's t-test: maternal weight gain, litter weight (calculated from the total weight of live foetuses per litter), foetal weight (calculated from the values for the mean per litter) and numbers of implantation sites, viable foetuses and corpora lutea; the ethanol group being used for comparison.

2. Indices: Pre- and post-implantation losses were analysed after calculation (for each litter separately) from the following equations:

$$\% \text{ pre-implantation loss} = \frac{\#\text{ corpora lutea} - \#\text{ implantations}}{\#\text{ corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\#\text{ implantations} - \#\text{ live fetuses}}{\#\text{ implantations}} \times 100$$

3. Historical control data: No historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: From p. 14: "Twenty-one animals died or were killed prematurely during the course of the study. The deaths or reasons for the premature killing of a total of eleven animals in the 0.5% v/v Tween 80 and 5% v/v ethanol control, 0.001 and 0.002 mg/kg/day groups were unrelated to treatment. The remaining ten animals receiving 0.005 mg/kg/day which died or were killed were all found to have internal haemorrhage (generally from the gravid uterus and/or thoracic contents). In surviving animals, however, there was no evidence of any adverse effects of brodifacoum

with the possible exception of a small subcutaneous haemorrhage in one doe receiving 0.002 mg/kg/day.

2. Body Weight: From p. 14: "Mean bodyweight gain during pregnancy was low in all groups although comparable to that seen in previous teratogenicity experiments in this laboratory using the Dutch rabbit. There was no evidence of any group differences."

TABLE 4 Maternal Body Weight Gain (g)[a]

Interval	Dose in mg/kg/day				
	0 (0.5% Tween 80)	0 (5% ethanol)	0.001	0.002	0.005
Pretreatment: Days 0 - 6	0.07	0.11	0.00	0.06	0.01
Treatment: Days 6 - 18	-0.02	0.02	0.03	0.01	0.10
Posttreatment: Days 18-29	-0.04	0.08	0.04	0.04	-0.05
Overall: Days 0 - 29	0.01	0.21	0.07	0.11	0.06

a Data calculated from information in Table 4 of report CTL/P/459 (p. 20 of MRID 00052442).

3. Gross Pathology: From p. 15: "The macroscopic findings in the pregnant does surviving to Day 29 did not reveal any treatment-related effects. However, the mortalities...[in the 0.005 mg/kg/day group]...indicated that brodifacoum caused hemorrhage, particularly from the gravid uterus."

4. Cesarean Section Data: From p. 15: "The administration of brodifacoum had no adverse effect on any of the parameters measured, apart from the number of pregnant does surviving to Day 29 in the 0.005 mg/kg/day group."

B. DEVELOPMENTAL TOXICITY

TABLE 5 Cesarean Section Observations[a]

Observation	0 (0.5% Tween 80)	0 (5% ethanol)	0.001 mg/kg/day	0.002 mg/kg/day	0.005 mg/kg/day
Number of females mated	15	15	15	15	15
Number of females pregnant	12	11	13	13	12
Number of pregnant females surviving to Day 29	11	8	10*	11	3
Mean number of implantations per litter	7.3 (2.0)	7.1 (2.3)	6.5 (2.6)	6.4 (2.0)	7.3 (2.1)
Resorptions: No. and %: early No. and %: late No. of litters with resorptions	6 7.5 1 1.3 5/11	7 12.3 1 1.8 3/8	13 20.0 0 0.0 8/10	7 10.0 1 1.4 4/11	2 9.1 0 0.0 2/3
Mean number of corpora lutea	8.4	8.0	7.9	7.4	7.7
Mean % pre-implantation loss	14.8 (12.7)	14.7 (14.5)	19.1 (23.5)	16.7 (16.5)	5.6 (9.6)
Mean % post-implantation loss	8.7 (13.0)	12.7 (23.5)	24.6 (22.9)	11.9 (17.6)	7.9 (6.8)

* One pregnant female which died on Day 29 not included in this value.
Standard deviations are given in parenthesis.
No statistically significant differences were found between the ethanol control and the other groups.

[a]Extracted from Table 6a (p. 22) of MRID 00052442 and Appendix D (p. 20-29) of MRID 40307201.

TABLE 6 Cesarean Section Observations[a]

Observation	0 (0.5% Tween 80)	0 (5% ethanol)	0.001 mg/kg/day	0.002 mg/kg/day	0.005 mg/kg/day
Number of live fetuses	73	49	52	62	20
Number of male fetuses	37	27	30	38	10
% male fetuses	50.7	55.1	57.7	61.3	50.0
Mean foetal weight (g)	32.8 (5.8)	37.7 (4.6)	36.9 (3.7)	37.6 (6.4)	35.3 (8.4)
Mean litter weight (g)	218.3 (87.3)	221.3 (68.1)	191.6 (99.7)	(208.0) (82.9)	238.7 (85.7)

Standard deviations are given in parenthesis.
No statistically significant differences were found between the ethanol control and the other groups.

[a]Extracted from Table 6b (p. 23) of MRID 00052442.

B. DEVELOPMENTAL TOXICITY

No treatment-related differences between the treated and control groups were observed for either the external/visceral or the skeletal examinations. There were statistically significant differences for specific observations, however, there was no dose-response.

TABLE 7 Intergroup Comparison of Soft Tissue Abnormalities[c]

Observation	Dose Level of Brodifacoum (mg/kg/day)				
	0 (0.5% Tween 80)	0 (5% etha-nol)	0.001	0.002	0.005
Number of fetuses processed	37	24	28	32	10
Brain Slight dilation of lateral ventricles	0	0	0	4[b]	0
Thorax Excess blood present	0	0	1	0	0
Heart Large clot in left atrium	0	0	1	0	0
Heart and great vessels abnormal[+]	0	0	1	0	0
Gonads Left ovary higher in abdomen than normal	0	1[a]	0	0	0
Kidneys Left-small	0	1[a]	0	0	0
Pelvic dilatation	1	1	0	0	0
Bladder and Ureters Distended with urine (associated with no urethral opening)	0	1	0	0	0

[+] Heart generally enlarged but particularly the right atrium; ventricles rounded and distended with blood, while the left atrium was small and empty. The interventricular septum and ventricular wall were patent. The aorta and pulmonary vessels were poorly developed.

[a] Same fetus

[b] Three fetuses from one litter affected.

[c] Extracted from Table 7, p. 24 of MRID 00052443

No statistically significant differences were found between the ethanol control and the other groups.

TABLE 8 SKELETAL EXAMINATION

Variant	Dose Level of Brodifacoum (mg/kg/day)				
	0 (0.5% Tween 80)	0 (5% etha-nol)	0.001	0.002	0.005
Number of fetuses examined	36	25	24	30	10
	No. %	No. %	No. %	No. %	No. %
Skull					
Fontanelle slightly widened	2 5.6	1 4.0	0 0.0	2 6.7	1 10.0
Fontanelle moderately widened	0 0.0	0 0.0	0 0.0	1 3.3	0 0.0
Partially ossified bones: Parietals	3 8.3	2 8.0	0 0.0	4 13.3	0 0.0
Vertebrae					
Lumbar: 8					
Total number ossified:	0 0.0	0 0.0	0 0.0	3 10.0	0 0.0
39	1 2.8	0 0.0	0 0.0	0 0.0	0 0.0
41	0 0.0	0 0.0	0 0.0	0 0.0	1 10.0
42	1 2.8	0 0.0	1 4.2	1 3.3	0 0.0
43	2 5.6	4 16.0	2 8.3	2 6.7	0 0.0
44	5 13.9	4 16.0	6 25.0	4 13.3	1 10.0
45	21 58.3	6 24.0	11 45.8	12 40.0	4 40.0
46	6 16.7	9 36.0	4 16.7	10 33.3	3 30.0
47	0 0.0	0 0.0	0 0.0	1 3.3	0 0.0
Tail broken - not included	0 0.0	2 8.0	0 0.0	0 0.0	1 10.0
Ribs					
12th left short	0 0.0	1 4.0	0 0.0	0 0.0	0 0.0
13th left short	2 5.6	1 4.0	1 4.2	0 0.0	1 10.0
13th right short	1 2.8	0 0.0	0 0.0	1 3.3	0 0.0
13th bilaterally short	2 5.6	2 8.0	2 8.3	1 3.3	0 0.0
13th left long	0 0.0	0 0.0	1 4.2	2 6.7	0 0.0
13th bilaterally long	4 11.1	2 8.0	5 20.8	9[b] 30.0	1 10.0
13th one long, one short	1 2.8	0 0.0	1 4.2	2 6.7	0 0.0
12th and 13th left fused	0 0.0	0 0.0	1[a] 4.2	0 0.0	0 0.0
Number of fetuses with extra ribs	10 27.8	5 20.0	10[c] 41.7	15[c] 50.0	2 20.0
Number of litters affected	7 63.6	4 50.0	6 75.0	8 72.7	2 66.7

[a] This fetus is also included under 13th left long.

[b] Statistically significantly different at the 5% level (2x2 contingency tables) compared with the ethanol control group.

[c] Statistically significantly different at the 1% level (2x2 contingency tables) compared with the ethanol control group.

Extracted from Table 8, p. 25 of MRID 00052443

TABLE 8 (continued) SKELETAL EXAMINATION

Variant	Dose Level of Brodifacoum (mg/kg/day)				
	0 (0.5% Tween 80)	0 (5% etha-nol)	0.001	0.002	0.005
Number of fetuses examined	36	25	24	30	10
	No. %	No. %	No. %	No. %	No. %
Sternebrae					
Partially ossified:					
1	16 44.4	11 44.0	5 20.8	9 30.0	4 40.0
2	1 2.8	2 8.0	2 8.3	3 10.0	3 30.0
3	0 0.0	2 8.0	0 0.0	1 3.3	0 0.0
Not ossified:					
1	3 8.3	4 16.0	1 4.2	1 3.3	2 20.0
2	0 0.0	1 4.0	0 0.0	0 0.0	0 0.0
Abnormal					
2	0 0.0	0 0.0	1 4.2	1 3.3	0 0.0
3	0 0.0	2 8.0	0 0.0	0 0.0	0 0.0
4	0 0.0	1 4.0	0 0.0	0 0.0	0 0.0
Forelimb assessment of digits					
1	9 25.0	10 40.0	3[b] 12.5	16 53.3	4 40.0
2	10 27.8	6 24.0	6 25.0	8 26.7	2 20.0
3	9 25.0	9 36.0	15 62.5	5 16.7	2 20.0
4	8 22.2	0 0.0	0 0.0	1 3.3	2 20.0
Hindlimb assessment of digits					
1	25 69.4	21 84.0	17 70.8	23 76.7	6 60.0
2	5 13.9	1 4.0	6[b] 25.0	6 20.0	0 0.0
3	5 13.9	3 12.0	1 4.2	1 3.3	4 40.0
4	1 2.8	0 0.0	0 0.0	0 0.0	0 0.0
Astragalus					
Partially ossified	16 44.4	9 36.0	9 37.5	8 26.7	4 40.0

[b] Statistically significantly different at the 5% level (2x2 contingency tables) compared with the ethanol control group.

Extracted from Table 8 (continued), p. 26 of MRID 00052443

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: From p. 5: "Brodifacoum, when dosed to rabbits at 0.005 mg/kg/day, caused a high proportion of maternal deaths. Despite the maternal toxicity there was no evidence of any teratogenic, embryotoxic or foetotoxic effect at this dose level. No treatment-related effects were seen in either the mother of the developing foetus at the two lower dose levels."

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: There was conclusive indications of maternal toxicity at the highest dose level (0.005 mg/kg/day), as only 3/12 pregnant females survived to Day 29. One of the major causes of death was uterine hemorrhage, consistent with the anti-coagulant activity of brodifacoum. In addition, prothrombin time in the preliminary study was significantly elevated in the 0.005 mg/kg/day animals relative to their controls (it is unfortunate that this study did not include data from animals dosed at 0.002 mg/kg/day, and prothrombin time was measured in only one female at 0.001 mg/kg/day). While the statement is made that there was no evidence of fetotoxic effects at even 0.005 mg/kg/day, it is noted (see p. 17 of MRID 40307201) that for animal #47 (died on day 16) "all implants have a haemorrhagic appearance." It is also noted that the numbers of available fetuses (20) and litters (3) in the 0.005 mg/kg/day group were relatively low. The numbers of available fetuses (62) and litters (11) in the 0.002 mg/kg/day group were also lower than this reviewer would normally like to see, and the fetuses were evaluated either for soft-tissue abnormalities (32 in the 0.002 mg/kg/day group) or skeletal abnormalities or variations (30 fetuses), rather than both. However, this study does give a lower NOEL for fetotoxicity (0.002 mg/kg/day) than the rat developmental toxicity study (0.02 mg/kg/day).

2. DEVELOPMENTAL TOXICITY: There were indications of developmental toxicity (hemorrhagic appearance in the implants of a female that died on day 16) in this study at 0.005 mg/kg/day. In addition, there were insufficient numbers of fetuses (20) and litters (3) available for examination at this dose level at 29 days. Therefore, the NOEL for developmental toxicity for brodifacoum is 0.002 mg/kg/day in the rabbit.

C. STUDY DEFICIENCIES AND ITS ADEQUACY: This study was previously reviewed (along with a rat developmental study) by Dr. Zendzian (Oct. 26, 1989; Caswell file document 007588). In that review, it was stated: "The reports remain unacceptable... individual observations on the fetal skeletons are missing from both supplements" [which would include the rabbit study].

"Based on the data provided the studies will be, at best, supplementary. Due to the cumulative toxicity of brodifacoum, maximum fetal exposure was not achieved until the end of and after the critical period of fetal development."

It is noted that MRID 40307202 includes (pages 14-19) individual macroscopic findings for all does in all groups. The mortalities in the 0.005 mg/kg/day group (pages 17-18) were associated with findings such as hemorrhage in the uterus ("Uterus: blood filled"), which are consistent with the anticoagulant activity associated with brodifacoum.

Although no individual observations on fetal skeletons are provided, the fetal incidence findings (refer to Table 8 in MRID 00052442, duplicated in the two sections of Table 8 of this DER) adequately demonstrate a lack of developmental toxicity involving skeletal development.

Although this reviewer has to express concern about the relatively low numbers of available fetuses (62) and litters (11) in the 0.002 mg/kg/day group, and the fetuses were evaluated either for soft-tissue abnormalities (32 in the 0.002 mg/kg/day group) or skeletal abnormalities or variations (30 fetuses), but not for both, the study gives a lower NOEL for fetotoxicity than the rat developmental study (rabbit: 0.002 mg/kg/day; rat: 0.05 mg/kg/day). The maternal NOEL in the rabbit study is 0.002 mg/kg/day, while in the rat study it is 0.001 mg/kg/day (but this is based on the equivocal finding of blood in the uterus of one 0.01 mg/kg/day rat, as well as in three rats at the 0.02 mg/kg/day dosage level.

It is concluded that the data in MRIDs 00052442 and 40307201 provide sufficient information to adequately define the maternal and fetal toxicity potential of brodifacoum in the rabbit.