



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

0 C11967

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

JUN 28 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Brodifacoum Acute Inhalation Toxicity Study

FROM: Byron T. Backus, Ph.D., Toxicologist *Byron T. Backus*
Toxicology Branch 2
HED (7509C) 6/27/96

TO: PM 81 Briscoe/Rubis
SRRD (7508W)

THROUGH: K. Clark Swentzel *K. Clark Swentzel* 6/27/96
Section Head, Review Section II
Toxicology Branch 2
HED (7509C)

and

Stephanie Irene, Ph.D., Acting Branch Chief
Toxicology Branch 2
HED (7509C) *Stephanie Irene* 6/27/96

DP Barcode: D201024

Submission: S461723

Chemical: 112701 (Brodifacoum; Talon)

Action Requested: "Please review study for guideline 81-3, MRID 43110501."

EXECUTIVE SUMMARY:

In an acute inhalation toxicity study (MRID 43110501) groups of young adult Wistar-derived (Alpk:APfSD strain) rats, 5/sex, were exposed (nose-only) for 4 hours to aerosols of brodifacoum (96.1% a.i.) generated from an acetone solution. The mean particulate concentrations were 0.82, 1.88 or 4.96 $\mu\text{g/L}$; corresponding brodifacoum concentrations were 0.69, 1.72 and 4.40 $\mu\text{g/L}$. The mass median aerodynamic diameters were 0.80, 0.89, and 0.68 μm , and the geometric standard deviations were 3.09, 1.91 and 2.54, respectively. Animals were observed for 14 days after exposure.



Recycled/Recyclable
Printed with Soy/Canola Ink on paper that
contains at least 50% recycled fiber

Inhalation LC₅₀ Males = 4.86 µg/L (based on particulate concentration)

Females = 3.05 µg/L

Combined = not reported

Brodifacoum is in TOXICITY CATEGORY I (inhalation LC₅₀ at or below 50 µg/L), based on LC₅₀ values in both sexes.

Mortalities (accompanied by symptoms consistent with anti-coagulant activity) occurred on days 4, 5 and 6 in 3/5 males and 5/5 females exposed to the highest concentration.

This acute inhalation study is classified as acceptable. It does satisfy the guideline requirement for an acute inhalation study (81-3) in the rat for technical brodifacoum.

BRODIFACOUM

ACUTE INHALATION STUDY (81-3)

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus, Date 6/27/96
Review Section 2, Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: K. C. Swentzel K. C. Swentzel, Date 6/27/96
Review Section 2, Toxicology Branch 2 (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Acute Inhalation Toxicity - rat [81-3]

DP BARCODE: D201024

SUBMISSION CODE: S461723

P.C. CODE: 112701

TOX. CHEM. NO.: 114AAA

MRID NO.: 43110501

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

CITATION: Parr-Dobrzanski, R. J. (1993). Brodifacoum: 4-Hour Acute Inhalation Toxicity Study in the Rat. ZENECA Central Toxicology Laboratory, U.K. Report No. CTL/P/4065; Study No. HR2213, June 23, 1993. MRID 43110501. Unpublished.

SPONSOR: Zeneca Inc.
Agricultural Products
Wilmington, DE 19897

EXECUTIVE SUMMARY:

In an acute inhalation toxicity study (MRID 43110501) groups of young adult Wistar-derived (Alpk:APfSD strain) rats, 5/sex, were exposed (nose-only) for 4 hours to aerosols of brodifacoum (96.1% a.i.) generated from an acetone solution. The mean particulate concentrations were 0.82, 1.88 or 4.96 µg/L; corresponding brodifacoum concentrations were 0.69, 1.72 and 4.40 µg/L. The mass median aerodynamic diameters were 0.80, 0.89, and 0.68 µm, and the geometric standard deviations were 3.09, 1.91 and 2.54, respectively. Animals were observed for 14 days after exposure,

Inhalation LC₅₀ Males = 4.86 µg/L (based on particulate concentration)
Females = 3.05 µg/L
Combined = not reported

Brodifacoum is in TOXICITY CATEGORY I (inhalation LC₅₀ at or below 50 µg/L), based on LC₅₀ values in both sexes.

Deaths (accompanied by symptoms consistent with anti-coagulant activity) occurred on days 4, 5 and 6 in 3/5 males and 5/5 females exposed to the highest concentration. There were no deaths among

BRODIFACOUM

ACUTE INHALATION STUDY (81-3)

animals exposed to lower concentrations.

This acute inhalation study is classified as acceptable. It does satisfy the guideline requirement for an acute inhalation study (81-3) in the rat for technical brodifacoum.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS:

1. Test Material: Brodifacoum
Description: off-white solid
Lot/Batch #: Y00052/038
Purity: 96.1% w/w a.i.
CAS #: 56073-10-0
Structure: n/a

Verification of concentration/homogeneity as necessary

2. Vehicle:

"Due to the known toxicity of brodifacoum and the physical characteristics of the technical material, it was not possible to generate atmospheres from the material as supplied. Atmospheres were generated from a solution of brodifacoum technical material in acetone at a final concentration of 5 mg/mL."

3. Test animals: Species: rat
Strain: Alpk:APfSD (Wistar-derived)
Age and weight at study initiation: "young adults"
males: 273-332 g; females: 215-234 g.
Source: from a colony maintained at Alderley Park.
Acclimation period: "a minimum of 5 days prior to exposure."

B. STUDY DESIGN AND METHODS:

1. Exposure conditions:

"Animals were exposed nose-only in restraining tubes...which were inserted into an ICI-designed PERSPEX exposure chamber...once the target concentration had been achieved and shown to be acceptably stable over approximately 30 minutes."

2. Animal assignment and treatment - Rats were allocated to three exposure groups as noted in Table 1, using a randomized block design, so that there were 5 males and 5 females in

each group. Rats were exposed (nose only) to Brodifacoum for a single 4-hour period. They were observed eight times during exposure (at approximately 30-minute intervals), immediately after exposure, and at least once a day thereafter for 14 days after exposure. Rats were weighed on days 1, 2, 3, 8 and 15. Survivors were sacrificed and a necropsy was performed.

TABLE 1. Concentrations, exposure conditions, mortality/animals exposed

Nominal Conc. ($\mu\text{g/L}$)	Particulate Conc. ($\mu\text{g/L}$)	Analytical Conc. ($\mu\text{g/L}$)	MMAD μm	GSD	Males	Females	Combined
1.05	0.82	0.69 ± 0.35	0.80	3.09	0/5	0/5	0/10
3.50	1.88	1.72 ± 0.16	0.89	1.91	0/5	0/5	0/10
10.35	4.96	4.40 ± 0.52	0.68	2.54	3/5 (days 4-6)	5/5 (days 4-6)	8/10

MMAD = Mass Median Aerodynamic Diameter
GSD = Geometric Standard Deviation

3. Generation of the test atmosphere and description of the chamber: "Atmospheres were generated from a solution of brodifacoum technical material in acetone at a final concentration of 5 mg/mL."

"Each atmosphere was generated using a glass concentric-jet atomiser. The stock solution containing the test substance was pumped to the atomiser using a Hamilton Microlab M fitted with a 50 μL syringe. Clean, dry air...was passed through the atomiser at flow rates of 13 liters/minute [the two highest-dose groups] or 14 liters/minute [the lowest dose group] at normal temperature and pressure and carried the atmospheres to the exposure chambers where they were further diluted by addition of air in order to maintain the target concentrations."

Time to equilibrium was not stated [Measurements of total particulate concentration and brodifacoum concentration taken at 0-3 minutes after initiation of exposure indicate that equilibrium had been attained - refer to Table 1A p. 26 of the report].

Analytical chemistry:

011967

Test atmosphere concentration:

From p. 15 of the report: "Particulate concentrations of the test atmospheres close to the animals' breathing zone, were measured gravimetrically, at approximately half-hourly intervals during exposure. This was done by drawing the test atmosphere, at a known flow rate for a known time, through a 25mm diameter Vinyl Metrical (VM-1) filter housed in a Delrin open-faced filter holder (filters and holders both supplied by Gelman Sciences Limited...). The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{concentration (mg/L)} = \frac{\text{post wt.} - \text{pre wt.}}{\text{time (min)} \times \text{airflow (Liters/min)}}$$

pre wt. = weight of filter prior to sampling (mg)
post wt. = weight of filter after sampling (mg)

From p. 16: "The atmospheric concentration of brodifacoum was determined by dissolving the formulation deposited on the VM-1 filters and the stages of the Cascade Impactor in ethanol and then diluting with acetonitrile where necessary. The resultant solutions were then analysed by liquid chromatography to calculate the atmospheric concentrations of brodifacoum."

Results are in table 1 above.

Particle size determination

From p. 15: "The aerodynamic particle size of each test atmosphere was measured by means of a Marple Cascade Impactor...which aerodynamically separates airborne particles into pre-determined size ranges. The amount of aerosol, by weight, in each size range, was then used to calculate the aerodynamic particle size distribution of the aerosol... the data was transformed using a log/probit transform and a linear regression derived from the cumulative data."

"Using this regression line, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated."

Results are in table 1 above.

4. **Statistics** - The LC_{50} was estimated by logistic regression. "Confidence limits were calculated using a likelihood ratio interval..."

5. Signed and dated GLP, quality assurance, and no claim of confidentiality statements are present.

C. RESULTS AND DISCUSSION:

1. Mortality is given in table 1. The Mortalities which occurred (8/10 rats of the highest exposure group; no mortalities at lower exposure levels) were on days 4-6 for both sexes.
2. Clinical observations - these were consistent with the known anticoagulant activity of brodifacoum (subcutaneous hemorrhage of the head and/or thorax, signs of bleeding from hind limbs and/or snout, decreased activity, increased respiratory depth, reduced respiratory rate and shaking).
3. Body Weight - "A small initial bodyweight loss was seen in animals from all exposure groups...attributable to the use of restraint during exposure. All surviving animals had gained weight by day 3 or day 8 and continued to gain weight throughout the remainder of the study."
4. Necropsy - From information on p. 22 the effects seen (enlarged and/or discolored thymuses, hemorrhage in the thoracic cavity of one male etc.) in animals killed in extremis or found dead were consistent with anticoagulant activities. Animals which survived the observation period showed no treatment-related effects at termination.

The LC_{50} (95% C.I.) for males is $4.86 \mu\text{g/L}$ (2.97-11.1)
females is $3.05 \mu\text{g/L}$ (1.88-4.96)
combined is not given.

D. CLASSIFICATION: Acceptable

E. STUDY DEFICIENCIES are as follows:

- Lack of a solvent control group
- Lack of a reporting of the LC_{50} in terms of the analytical (actual brodifacoum) concentration.

These are minor deficiencies. The test material is extremely toxic by the inhalation exposure route, with an inhalation LC_{50} value considerably below $50 \mu\text{g/L}$ ($=0.05 \text{ mg/L}$). Brodifacoum technical then is in toxicity category I in terms of its inhalation exposure hazard (although it is noted it was necessary to dissolve the Brodifacoum technical in acetone in order to get it into a form which could be tested by the inhalation exposure route).