MEMORANDUM

SUBJECT: Brodifacoum, Rodenticide

TO: William H. Miller PM-16
    Registration Division (TS-767)

FROM: Robert P. Zemtian PhD, Head
    Review Section III
    Toxicology Branch
    HED (TS-769)

THROUGH: William Burnam, Chief
    Toxicology Branch

Compound: Brodifacoum

Formulation: TALON-G Rodenticide Pellets

Registration #: 10182-L1

Accession #: 250077, 255704 & 252894

Tox Chem #: 114AAA

Registrant: ICI Americas Inc.

Action Requested

The Registrant requests an amendment of the registration of TALON-G to allow outdoor use against commensal rodents.

Recommendation

Toxicology Branch recommends that the amendment be denied. The Branch further recommends that additional testing of brodifacoum be required and that no further registration be approved until they are completed. Tests required are, 1) an antidotal study in dogs, 2) a special metabolism study to evaluate the long half-life and potential for bioaccumulation, and 3) an additional teratology study(s).
Brodifacoum is a highly toxic anticoagulant which has a demonstrated half-life in mammalian species of at least six months. This property can lead to one or more of three extremely serious adverse toxic effects. 1. Bioaccumulation can be expected to occur so that toxic responses can occur at intervals between doses as great as six months. 2. Under conditions of stress tissue stored compound can be mobilized and toxicity occur months after dosing. 3. An individual acutely poisoned with this compound will require treatment of extensive duration compared to commonly known anticoagulants of this type such as warfarin.

**Chemistry**

![Chemical structure of Brodifacoum]

**Background**

Brodifacoum is an extremely toxic anticoagulant rodenticide which functions as a vitamin K, antagonist. The original toxicity data on the technical material, a 0.25% liquid concentrate and a 0.0005% w/v pellet dosage formulation were summerized in an EPA memo by R.A. Hessert (8/2/78). The acute oral toxicity of the technical material is as follows.
Male Rat LD$_{50}$ 0.27mg/kg  Guinea pig LD$_{50}$ 2.87mg/kg
Mouse LD$_{50}$ 0.4mg/kg  Dog LD$_{50}$ 0.25 to 1mg/kg
Rabbit LD$_{50}$ 0.29mg/kg  Sheep LD$_{50}$ 25mg/kg
Cat LD$_{50}$ 25mg/kg

Gassert's comment on the antidotal study submitted with the toxicity data is quoted in full as follows:

"Antidote Studies: Vitamin K$_1$ is antidotal. The minimum dose of Vitamin K$_1$ required to antidote a massive acute dose of the rodenticide lies between 10 and 20 mg/kg."

Without information on the experimental animal used, the dose of compound, the dose(s) of Vitamin K$_1$ and the criteria for effectiveness utilized it is impossible to use the statement in the Gassart memo as a basis for treatment.

Subsequently Doherty (9/12/78) approved an EUP for a 0.005% bait formulation for use in orchards on the basis of acute studies submitted with the request.

Doherty (10/19/78) recommended approval of a registration of the 0.005% bait formulation on the basis of the data reviewed in his 9/12/78 memo.

Doherty (10/4/79, two memos) reviewed the label for technical and 0.25% concentrate brodifacoum and noted the lack of teratology studies on the compound.

Doherty (6/18/80) reviewed rat and rabbit teratology studies on the technical material. Both studies were negative.

For the rat study Doherty concluded;

"This study is Core Minimum. No positive control was included. The raw data are not included and data are in summary tables only."

For the rabbit study Doherty concluded;

"This study is Core Minimum. No positive control was included. The data are presented in summary tables only. No raw or individual animal data as (sic) presented."

The studies were improperly classified, a lack of individual animal/litter data in teratology reports makes them impossible to review and the studies should have been classified invalid.
Biscardi (7/7/81) reviewed an antidotal study in the beagle dog. Since all dogs dosed with Brodifacoum were treated with the antidote vitamin K₁, it was impossible to determine if a toxic dose of the anticoagulant had been given. Therefore, it was impossible to determine if vitamin K₁ has protected against any toxic effect. Dr. Biscardi classified this study invalid.

Additional Information

The Registrant has submitted the following additional studies which have been reviewed with the noted conclusions. DERs are attached.

Citation


Conclusion

Following an oral dose of 0.25mg/kg, brodifacoum is almost completely absorbed and only very slowly excreted in the urine. The half-life is estimated as 150-200 days with at least 50% of the retained radioactivity represented by the parent compound.

Citation

WBA 8119: Acute Oral Toxicity. G.R. Parkinson, Central Toxicology Laboratory. Imperial Chemical Industries Ltd. CTL/P/216 (revised). Jan 1976

Conclusion

Acute oral toxicity in dogs was between .25 and 1.0 mg/kg and in cats about 25mg/kg.

Citation


Conclusion

The LD₅₀ was estimated as between 3.5 and 2.0 mg/kg.
Citation

PP 581: Acute Oral Toxicity to Sheep, G.R. Parkinson, Central Toxicology Laboratory, Imperial Chemical Industries Ltd, CTL/P/258. Sept 1976

Conclusion

LD50 was estimated as in excess of 25mg/kg.

Discussion

The coumerin type anticoagulants, as exemplified by Warfarin, have properties that make them particularly useful as rodenticides. A single properly chosen dose is not lethal to the rat or nontarget organisms such as dogs, cats or children. Repeated daily dosing by the rat is lethal. The safety to nontarget organisms lies in the small likelihood that they will ingest several doses of the anticoagulant at frequent enough intervals to produce toxic effects. This is a function of the half-life of Warfarin, between one and two days, which promotes bioaccumulation at shorter intervals and makes bioaccumulation impossible at longer intervals. In addition the treatment of this type of toxicity, with vitamin K and blood transfusions, is well established and usually successful.

Brodifacoum differs from warfarin in that it is considerably more toxic, a single feeding of the bait formulation will kill a rat, and it has a half-life that is at least 50 times longer. The treatment of brodifacoum toxicity has not been experimentally established but one may expect that it would require an extremely expended duration compared to warfarin.

For these reasons it is necessary to have a fuller understanding of the metabolism of the compound. A proposed protocol for the metabolism of rodenticides in enclosed which will provide the information required.

An antidotal study in dogs is required with proper controls. The study should include at least 2 animals/sex/group. Groups should consist of, 1) Treated with an acutely lethal dose. 2) Treated with an acutely lethal dose and then treated with antidotal doses at 24 hour intervals until the group one animals have died. 3) Treated with an acutely lethal dose and then treated with antidotal doses starting when toxic signs are observed and continued until complete recovery. Blood coagulation parameters should be followed on all animals.

The teratology studies submitted raise serious questions since warfarin and several other coumerin type antivitamin
anticoagulents are teratogenic. Warfarin has been demonstrated to be teratogenic in humans. In general all antivitamins are teratogenic under proper experimental conditions. Since the detailed results of the teratology studies were not submitted it is impossible to properly review the studies. At least one additional teratology study is required in a species which has been shown to be sensitive to warfarin.

Enclosure

Metabolism Studies for Rodenticides, Proposed by Robert P. Zendzian PhD, Pharmacologist, Toxicology Branch, July 1984

References


Doherty, J., MEMO, EPA Registration No. 10182-EA. (Rodenticide Pellets) and 10182-ER (Rodenticide Bait Pack), Oct 19, 1978


Doherty, J., MEMO, EPA File Symbles 10182-EN/EA/ER/EL/EU/EG/EE. Products Containing 0.005% Brodifacoum. Oct 4, 1979

Biscardi, S., MEMO, A One Month Anticoagulant Antidote Study in Beagle Dog FINAL REPORT. Jul 7, 1981.
Metabolism Studies for Rodenticides

Introduction

This study is designed to determine the persistence and potential for bioaccumulation of rodenticides in the mammalian species. A rodenticide which persists in the body of a nontarget organism presents a significant danger in that it offers the possibility for chronic poisoning with long intervals between doses and can significantly increase the duration and complexity of treatment required in cases of acute poisoning.

When Required

This study is required for the initial registration of all rodenticides.

Materials

An analytically pure sample of the rodenticide isotopically-labeled in a portion of the molecule that will be indicative of the presence of the molecule in the organism should be used. Labeling may not be needed if specific and sensitive physical-chemical methods exist for identifying the compound.

Young adult male and female rats of a commonly used strain are the species of choice. Other mammalian species may be used for particular compounds but a specific rationale for this choice must be supplied.

Methods

1) Route. The oral route should be used unless another route of exposure is expected to occur in accidental exposure. In some cases it may be necessary to study more than one route.

2) Dose. A single oral dose at one-tenth the LD₅₀ is recommended. The dose should not be expected to produce mortality. Since the oral LD₅₀ of compounds in the rat often differs by sex a different mg/kg dose may be necessary for each sex.

3) Number of Animals. At least five males and five females should be used.

4) Observation period. Animals should be maintained individually in metabolism cages for seven days or until 90 percent of the dose is excreted, whichever comes first. Animals are killed at the end of the observation period.
5) Collection and analysis of excreta. Expired air, urine and feces should be collected at suitable intervals during the observation period and analyzed quantitatively for the test material. Intervals of 4, 8, 12 and 24 hours, 1, 2, 3, 4, 5, 6 and 7 days are suggested. Expired air need not be collected if data is available to show lack of excretion by this route during the first 24 hours after dosing.

6) Tissue distribution. At sacrifice, the quantity of labeled material remaining in bone, brain, fat, gonads, heart, kidney, liver lungs, muscle, spleen, other tissues which may be expected to retain the particular compound and residual carcass will be determined.

7) Metabolism. Identification of metabolites is not required unless significant bioretention occurs. Essentially if the half-life exceeds 48 hours or accumulation occurs in a particular organ, analysis should be performed to determine the quantity of active rodenticide retained.

8) Additional determinations. Blood kinetics are not usually required but may be useful if bioretention occurs.

Reporting

Reports should provide the information required in the Pesticide Assessment Guidelines, Subdivision F, Series 90-5 Reporting of data and Series 95-1 Metabolism study.

Proposed by

Robert P. Zendzian PhD
Pharmacologist
Toxicology Branch HED

July 1984
Data Evaluation Report

Compound: Brodifacoum

Citation

Reviewed by
Robert P. Zendzian PhD
Pharmacologist

Core Classification
Supplementary

Tox Category: N/A

Conclusion
Following an oral dose of 0.25mg/kg brodifacoum is almost completely absorbed and only very slowly excreted in the urine. The half-life is estimated as 150-200 days with at least 50% of the retained radioactivity represented by the parent compound.

Materials
Brodifacoum, analytical grade, 96% pure, cis:trans ratio 64.6:35.6

Brodifacoum, uniformly labeled on the phenyl ring of the coumarin moiety as individual isomers, mixed one to one for administration.

Hydroxycoumarin analytical grade

Adult male rats (190-210g) Alderly Park specific pathogen-free strain (Dister-Derived).

Methods

1) Three rats were dosed orally with 14C brodifacoum (6.6 uCi/kg; 0.25mg/kg) and urine and feces collected individually at 24 hour intervals for 10 days. At termination the rats were killed and abdominal fat, kidneys, heart, liver and residual carcasses collected for analysis.

2) Three rats were dosed orally with 14C brodifacoum (6.6 uCi/kg; 0.25mg/kg) and killed 10 days after dosing. Blood (12ml) was collected for analysis by cardiac puncture. Pancreas and spleen were also collected.

3) Three rats were dosed orally with 14C brodifacoum (6.9 uCi/kg; 0.5mg/kg) and urine and feces collected at 24 hour intervals for 5 days.
4) Three rats were dosed orally with 14-C brodifacoum (7.2 uCi/kg; 1.5mg/kg) and urine and feces collected at 24 hour intervals for 5 days.

5) One rats was dosed orally with 14-C brodifacoum (6.6 uCi/kg; 0.25mg/kg) and expired air collected for 48 hours.

5) The bile ducts were cannulated on three rats. After recovery from anesthesia the rats were dosed orally with 14-C brodifacoum (6.6uCi/kg; 0.25mg/kg) and the bile collected at 24 hour intervals for 48 hours.

7) Three rats (80-90gm body weight) were given a single oral dose of 14-C brodifacoum (6.6uCi/kg; 0.25mg/kg). A single rat killed, quick frozen, block embedded and sectioned (longitudinal saggital sections) for whole body autoradiography 1, 5 and 10 days after dosing.

8) Twenty-four rats were dosed orally with 14-C brodifacoum (6.0uCi/kg; 0.21mg/kg) and killed in groups of 3 at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hours after dosing. Blood (2 ml) was taken by cardiac puncture. Blood was also collected from 3 untreated rats.

9) Six rats were dosed orally with 14-C brodifacoum (6.0uCi/kg; 0.21mg/kg) and killed in groups of 3 at 10 and 17 hours after dosing. Blood (2 ml) was taken by cardiac puncture.

Samples of urine, feces, bile and extracts from various organs were analysed for metabolites by several chromatographic procedures.

Results

1) Following an oral dose of 0.25mg/kg, the mean percent of dose excreted in the urine was 4.31 after 10 days with the highest excretion on day one (0.88) and excretion stabilizing on days 5 to 10 at 0.02 to 0.04 %/day. In the feces the mean percent of dose after 10 days was 11.01 with the highest excretion on day 2 (4.70%). Fecal excretion decreased throughout the 10 day collection period.

The mean percent of dose remaining in various tissues after ten days was abdominal fat 2.29, liver 22.34, kidney 3.79, heart 3.10 and residual carcass 80.32.

2) Following an oral dose of 0.25mg/kg, the mean percent of dose percent in tissues after 10 days was pancreas 2.33, spleen 0.16 and blood 0.05.

3) Following an oral dose of 0.5mg/kg, the mean percent of dose excreted in the urine was 2.31 after 5 days with the highest excretion on day one (7.05%). In the feces the mean
percent of dose after 5 days was 30.75 with the highest
excretion on day one (14.95%). One rat died on day four and
the remaining two on day five.

4) Following an oral dose of 1.5mg/kg, the mean percent
of dose excreted in the urine was 2.81 after 5 days with the
highest excretion on day one (2.01%). In the feces the mean
percent of dose after 5 days was 42.56 with the highest
excretion on day two (16.23%). All three rats died on day
on day five.

5) Following a single oral dose of 0.25mg/kg to one rat
no radioactivity was detected in expired air over 48 hours.

6) Following an oral dose of 0.25mg/kg, the mean percent
of dose excreted in the bile was 0.55 for the first 24 hours
and 0.77 for the second with a total of 1.36.

7) Following an oral dose of 0.25mg/kg, whole body
autoradiograms at one, five and ten days post dose showed
that the highest concentrations of radioactivity were present
in the liver, pancreas and salivary glands with activity also
present in the gastric mucosa, intestinal mucosa, vertebrae,
nasal mucosa, kidneys, adrenals, meninges, fat and skins.

8 & 9) Mean blood concentrations of brodifacoum (ng
equivalents /ml of blood) following a single oral dose of
0.21mg/kg are given in the following table taken from table 4
of the report. Activity was less than the limit of detection
(1.1 ng eq/ml) on day 17.

Hrs after
dosing | 0.25 | 0.50 | 1.0 | 2.0 | 4.0 | 6.0 | 8.0 | 10.0 | 12.0 | 14.0 | 16.0 | 18.0 | 20.0 | 22.0 | 24.0 | 240.0
ng eq/ml | 2.8 | 2.2 | 14.4 | 5.9 | 10.1 | 11.4 | 15.4 | 15.1 | 13.4 | 6.7 | 5.3 | 1.3

Analysis for metabolites showed that 50% or more of the
retained radioactivity was represented by the parent compound.

Discussion

The dose of 0.25mg/kg brodifacoum administered in most of
these experiments approximates the LD50 in the male rat.
The compound is almost completely absorbed and only very
slowly excreted. The half-life is estimated as 150-200 days
with at least 50% of the retained radioactivity represented
by the parent compound. This extensive half-life indicates a
serious potential for cumulative toxicity.

This study satisfies the Guideline requirements for a
single high oral dose in male rats.
Data Evaluation Report

Compound: Brodifacoum. WBA 8119

Citation


Reviewed by:

[Signature]
Robert P. Zendzian PhD
Pharmacologist

Core Classification: Supplementary

Tox Category: I

Conclusion

Acute oral toxicity in dogs was between 0.25 and 1.0 mg/kg and in cats about 25 mg/kg.

Materials

WBA 8119 (brodifacoum)

Female beagle dogs (8-12 kg)

Male and female cats (2-3 kg)

Methods

Compound was dissolved in polyethylene glycol 300 and administered orally by gavage. Animals were observed for 4 weeks. Necropsies were performed on all animals killed in extremis or at termination. Doses used and results are shown in Tables 1 & 2.

Results

Table 1. Female Dogs

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0.25</th>
<th>0.50</th>
<th>1.00</th>
<th>1.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. died/no. treated</td>
<td>0/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
</tr>
</tbody>
</table>

LD50 estimated as between 0.25 and 1.3 mg/kg.

Toxic signs appeared at about six days in the fatalities consisting of subdued behavior, loss of appetite, pale, respiratory difficulties, hypothermia, pica, fasciculations and minor external hemorrhages. Necropsy of animals killed in extremis revealed hemorrhages, particularly in the neck and thorax.
Table 2. Male and Female Cats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>5.00</th>
<th>10.00</th>
<th>25.00</th>
<th>50.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>(no. died/no. treated)</td>
<td>0/2</td>
<td>0/2</td>
<td>1/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>

LD$_{50}$ estimated at 25mg/kg

The animal that died was not necropised. Animals at 50mg/kg showed subdued behavior and loss of appetite at about two weeks but were normal at 4 weeks.
Compound: Brodifacoum. WBA 8119

Citation:
The Acute Oral Toxicity of WBA 8119 to the Domestic Pig
Jan 27, 1976.

Reviewed by: Robert P. Zendzian PhD
Pharmacologist

Core Classification: Supplementary

Tox Category: I

Conclusion:
The LD50 was estimated as between 0.5 and 2.0 mg/kg.

Materials:
- WBA 8119 (brodifacoum)
- Large white pigs from a local pig breeder.

Methods:
Compound was administered orally by gravage in polyethylene
glycol 300. Two animals per dose were dosed at 0.5, 1.0, 2.0,
5.0 or 10 mg/kg. Animals were observed for 21 days. Animals
were sacrificed in extremis or at termination and necropsied.

Results:
All animals died except one at 0.5 and two at 2.0 mg/kg.
Deaths occurred 3 to 18 days postdose. LD50 was estimated as
between 0.5 and 2.0 mg/kg. Animals appeared normal until
about four hours before death. Some loss of muscle
function and mild convulsions were observed. Bleeding from
the nose, ears and rectum was observed immediately prior to death.
Necropsy revealed signs of internal bleeding in the animals
that died.
Compound  Brodifacoum, PP 581

Citation

PP 581: Acute Oral Toxicity to Sheep, G.R. Parkinson, Central Toxicology Laboratory, Imperial Chemical Industries Ltd. CTL/P/258. Sept 1976

Reviewed by Robert P. Zendzian PhD Pharmacologist 2/23/74

Core Classification  Supplementary
Tox Category  Could not be determined

Conclusion

LD₅₀ was estimated as in excess of 25mg/kg.

Materials

PP 581 (brodifacoum)

Castrated sheep (30-35kg)

Methods

Compound was administered orally by drench, in polyethylene glycol, to two sheep at 25mg/kg. Animals were observed for three weeks and then necropsied.

Results

No toxic effects were observed. LD₅₀ was estimated as in excess of 25mg/kg.