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

007588

OCT 26 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Brodifacoum, Teratology Studies and Other Data Requirements

TO: William Miller PM-16/5
Registration Division (H7505C)

FROM: Robert P. Zendzian Ph.D. 10/5/89
Senior Pharmacologist
SACB, HED (H7509C)

THROUGH: Albin Kocialski Ph.D. ABK 10/25/89
Head
Registration Standards and Special Review Section

Reto Engler Ph.D.
Chief
Science Analysis and Coordination Branch

Compound; Brodificoum — Tox Chem #114AAA

Registration #10182-28&29 — Registrant; ICI

MRID#403072-01&02 and #52442 & 52443 — Tox Project #9-1818

Action Requested

Review the following studies and comment on data requirements;

Brodifacoum: Teratology study in the Rabbit, M.C.E. Hodge, P.B. Banham, D. Richards & T.M. Weight, Central Toxicology Laboratory, Imperial Chemical Industries Limited, Report No. CTL/P/459, Jan 30, 1980, MRID # 52442

Brodifacoum: Teratology study in the Rabbit, Individual animal data supplement, Addendum to MRID#00052442, Study completed on Aug 12, 1980, ICI Central Toxicology Laboratory, CTL/P/459S, 8/13/87, MRID 403072-01

Brodifacoum: Teratology study in the Rat, M.C.E. Hodge, P.B. Banham, D. Richards, T.M. Weight & June Wilson, Central Toxicology Laboratory, Imperial Chemical Industries Limited, Report No. CTL/P/437, Jan 22, 1980, MRID #52443

Brodifacoum: Teratology study in the Rat, Individual animal data supplement, Addendum to MRID#00052443, M.H. Litchfield, Study completed on Jan 29, 1980, ICI Central Toxicology Laboratory, CTL/P/437S, 8/13/87, MRID 403072-02

Conclusions

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.

Recommendations

It is recommended that the teratology studies be repeated in both rabbit and rat with dosing to start at the time of mating. Due to maternal lethality at the high dose (0.005 mg/kg/day), the new rabbit study should use 0.002 mg/kg/day as the high dose. The new rat study should use the same doses as the origional study.

A metabolism study in the rat is still required. Particular attention should be paid to determining the half life of brodifacoum.

An antidotal study in the dog is still required.

Background

In September 1984 Zendzian reviewed a request for an amendment of the Talon-G registration to allow outdoor use against commensal rodents. The active ingredient of Talon-G is brodifacoum. At that time it was recommended;

"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 (ies)."

The 1984 memo (copy attached) presents further background material which provides the basis for considering the reports of the teratology studies unacceptable.

It is not known if or when Registration Division conveyed this information to the Registrant.

On July 31, 1987 the Agency requested that the registrant label their brodifacoum manufacturing use products with a warning against exposure of pregnant women. The Agency explained: "The reason for these statements is the potential for anticoagulants to be teratogenic or to cause spontaneous abortions." "Because there is not a complete, acceptable data base available for brodifacoum which negates the need for one of these statements, the Agency believes that the label warning is prudent."

The Registrant responded, Aug 13, 1987, that the rat and rabbit teratology studies (MRID #s52442 & 52443 were negative and therefore the warnings are not required. The 1987 submission included the 'Individual Animal Data Supplements' listed above. This information was conveyed to HED on July 24, 1989.

Discussion

There are two issues involved in evaluating the potential for reproductive toxicity of brodifacoum, a reporting issue and a scientific issue. The reporting issue involves getting sufficiently complete reports of the rat and rabbit studies to allow scientific review and evaluation. The origional reports were deficient in that they lacked individual data which whould have allowed the Agency review scientist to evaluate the conclusions made in the report. Because of this deficiency they should not have been reviewed. As shown by the supplements, the data were readily available and could have been provided in a reasonable time after the origional submission, if the Agency had asked for them. Unfortionately, when the supplements were provided, and fowarded to HED for evaluation, they were still deficient in that 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. However, the submission now contains sufficient information to allow us to address the scientific issue of brodifacoum reproductive toxicity.

The coumerin type anticoagulants, and the second generation compounds such as brodifacoum, act as vitamin K antagonists which have their major toxic effect by inhibition of the production of several of the plasma clotting factors. The maximum response to a single dose occurs several days after dosing when the cotting factors in the blood have been suficiently reduced by normal loss. This response is dose dependent and ranges from clinical detectable increased clotting time through frank bleeding to death. This delay in maximum response also occurs following repeated dosing.

The prototype anticoagulant, Warfarin, is used as a human drug and has been show to be fetotoxic in humans. Teratogenic

response and/or fetal loss occur in pregnant women who are taking the drug chronically for cardiovascular problems. The clotting time of these individuals is significantly increased as a result of their treatment. This effect has also been demonstrated in experimental animals. In general antivitamins have been shown to be teratogenic when properly tested in experimental animals. The information in the rabbit study indicates that brodifacoum has not been properly tested in that the dosing was such that the maximum toxic response, maternal death at the high dose, occured at the end of and after the sensitive period of fetal development. We may reasonably assume that the same time maximum effect relationship was present at the lower doses.

The report and supplement of the rat study do not contain information on the toxicity to the dams. However, considering the mechanism of brodifacoum activity, we may reasonably assume the same time relationship of dose and maximum toxic response occurs in the rat and conclude that the fetuses were not dosed at the time of maximum response to the test compound.

Attachments

DERs

One-liners

Data Evaluation Report

Compound Brodifacoum

Citation
Brodifacoum: Teratology study in the Rabbit, M.C.E. Hodge, P.B. Banham, D. Richards & T.M. Weight, Central Toxicology Laboratory, Imperial Chemical Industries Limited, Report No. CTL/P/459, Jan 30, 1980, MRID # 52442

Brodifacoum: Teratology study in the Rabbit, Individual animal data supplement, Addendum to MRID#00052442, Study completed on Aug 12, 1980, ICI Central Toxicology Laboratory, CTL/P/459S, 8/13/87, MRID 403072-01

Reviewed by Robert D. Zendzian Ph.D. 10/18/89
Senior Pharmacologist

Core classification Report Unacceptable

Conclusions
The 'Individual Animal Data Supplement' lacks individual data on the skeletal examinations of the fetuses. The study cannot be evaluated. Ten of the fifteen females dosed with 0.005 mg/kg/day (days 6-18) died of compound-related toxicity on days 15-23 of gestation indicating that maximum toxicity occured at the end of and after the critical period of fetal sensitivity.

Materials

Brodifacoum, batch # 2,3,4,5,R1,
CTL reference # Y00052/002/001
Purity 92.5%

Nulliparous, female Dutch rabbits 1.62-3.15 kg at the start of the study supplied by Ranch Rabbits.

Experimental Design

Does were randomly assigned to the following test groups; The day of mating was day zero and does were dosed days 6-18 after mating.

Group	Dose mg/kg/day	Number Animals
1	Control (5% v/v aqueous ethanol)	15
2	0.001	15
3	0.002	15
4	0.005	15
5	control (0.05% v/v aqueous Tween 80)	15

Dosing suspensions were prepared from an ethanol solution of brodifacoum prepared so that the final solvent consisted of 5% ethanol (v/v) in water. Animals were dosed by oral gavage.

Animals were observed daily for toxicity and weighed on days 0, 6-18 (inclusive), 24 and 29 of gestation.

Animals were sacrificed on day 29 of gestation and necropsied. The uterus was removed and examined for resorptions and live fetuses. Fetuses were weighed and sexed. Fetuses were examined externally for abnormalities, half of each litter was examined for skeletal abnormalities and the remaining half for soft tissue abnormalities.

Results

Table 3 from the report presents the mortalities observed during the study. Figure 1 presents graphically the time of death of the animals given the high dose (0.005 mg/kg/day). The deaths at this dose are considered compound related. Ten of the fifteen females dosed with 0.005 mg/kg/day (days 6-18 of gestation) died of compound-related toxicity on days 15-23 of gestation indicating that maximum toxicity occured at the end of and after the critical period of fetal sensitivity.

The report concludes that "brodifacoum has no effect on embryonic or feotal development, even at a level that was lethal to a high portion of pregnant animals." Due to the lack of individual fetal data on the results of skeletal examination this conclusion could not be evaluated.

Discussion

The coumerin type anticoagulants, and the second generation compounds such as brodifacoum, act as vitamin K antagonists which inhibit the production of several clotting factors. Death occurs several days after a single lethal dose when the clotting factors in the blood have been sufficiently reduced by normal loss. As demonstrated in this study, repeated doses also have a lag between first dose and maximum toxic effect (death). This is a combination of the necessity of 'building an effective dose' and the period of normal loss of clotting factors. This lag is particularly important in a teratology study if the period of maximum toxicity occured at the end of the period of fetal sensitivity rather during that period. Starting dosing at the beginning of the 'normal' exposure period can be expected to have produced such a delayed maximum effect in this study. Thus, in this study, the early fetus was not dosed to the maximum extent that was possible under the dosing regimen and we do not have a true test of the fetal toxicity of the compound.

BRODIFACOUM: TERATOGENICITY STUDY IN THE RABBIT

TABLE 3

MORTALITY

Dose-level of Brodidacoum (mg/kg/day)	Number of Mortalities*	Probable Cause of Death or ill health
Control (5% v/v ethanol)	3	Dosing accident (2) abortion (1)
0.001	4	Dosing accident (1) unknown (1)
0.002	2	Dosing accident (2) unknown (2)
0.005	10	Internal haemorrhage(10)
Control 90.5% v/v Tween 80)	2	Abortion (1); Unknown (1)

* These numbers include animals which were killed because of ill-health

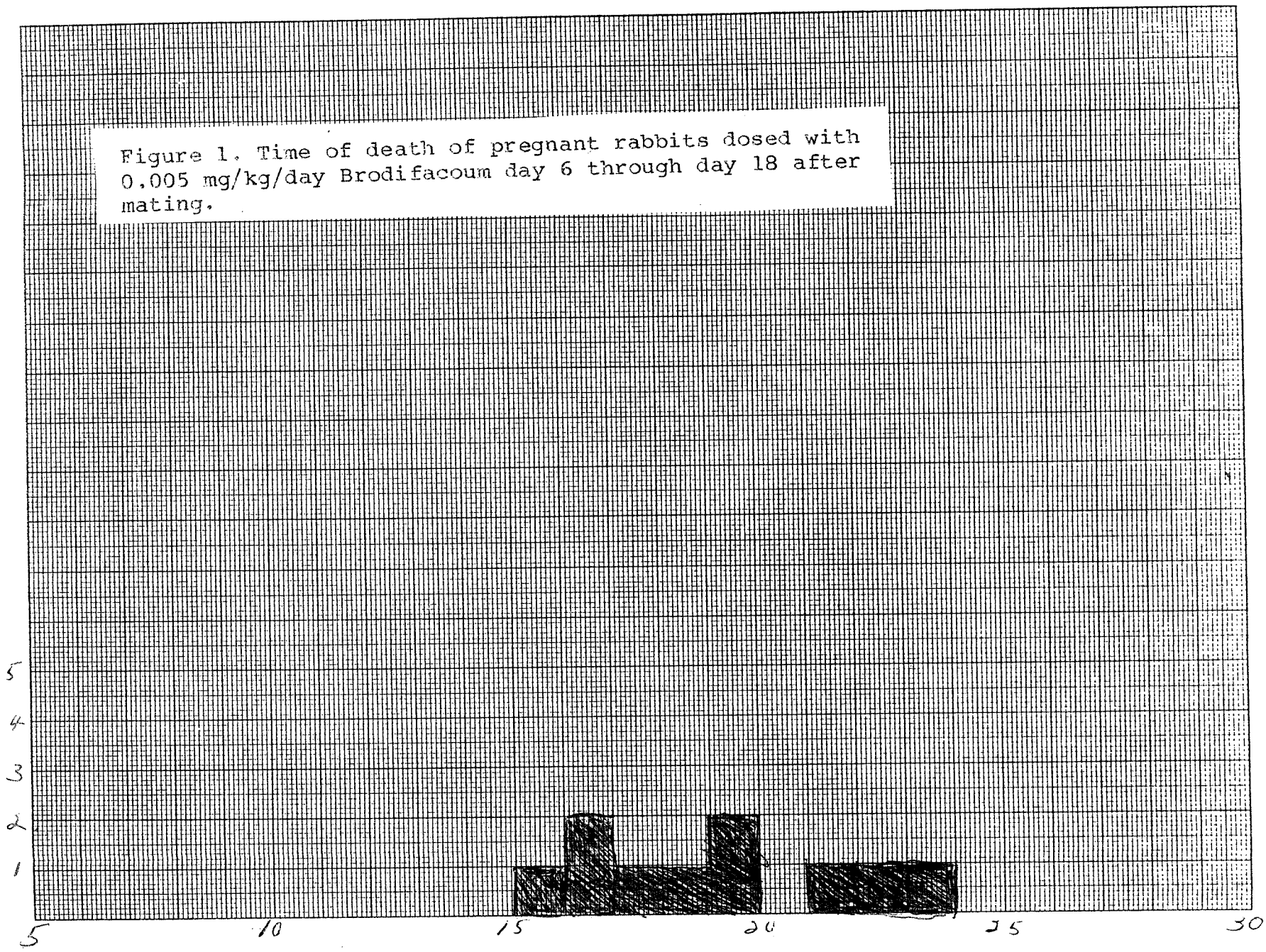
This is a true copy of the table.

18/10/89

Robert P. Zendzian Ph. D.
Senior Pharmacoalogist
Oct 18, 1989

NOTE: Probable cause of death for the 0.001 and 0.002 mg/kg/day groups are interchanged on this table. This information has been verified from the individual data supplement.

Figure 1. Time of death of pregnant rabbits dosed with 0.005 mg/kg/day Brodifacoum day 6 through day 18 after mating.



Data Evaluation Report

Compound Brodifacoum

Citation
Brodifacoum: Teratology study in the Rat, M.C.E. Hodge, P.B. Banham, D. Richards, T.M. Weight & June Wilson, Central Toxicology Laboratory, Imperial Chemical Industries Limited, Report No. CTL/P/437, Jan 22, 1980, MRID # 52443

Brodifacoum: Teratology study in the Rat, Individual animal data supplement, Addendum to MRID#00052443, Study completed on Jab 29, 1980, ICI Central Toxicology Laboratory, CTL/P/437S, 8/13/87, MRID 403072-02

Reviewed by Robert P. Zendzian Ph.D. 10/18/89
Senior Pharmacologist

Core classification Report Unacceptable

Conclusions
The 'Individual Animal Data Supplement' lacks individual data on maternal toxicity and on the skeletal examinations of the fetuses. The study cannot be evaluated.

Materials

Brodifacoum, batch # 2,3,4,5,R1,
CTL reference # Y00052/002/001
Purity 92.5%

Nulliparous, female Wistar-derived rats from the colony as Alderly Park

Experimental Design

Females were randomly assigned to the following test groups; The day of mating was day zero and females were dosed days 6-15 after mating.

Group	Dose mg/kg/day	Number Animals
1	Control (5% v/v aqueous ethanol)	30
2	0.001	30
3	0.01	30
4	0.02	30

Dosing suspensions were prepared from an ethanol solution of brodifacoum prepared so that the final solvent consisted of 5% ethanol (v/v) in water. Animals were dosed by oral gavage.

Animals were observed daily for toxicity and weighed on days 0, 6-15 (inclusive) and 21 of gestation.

Animals were sacrificed on day 21 of gestation and necropsied. The uterus was removed and examined for resorptions and live fetuses. Fetuses were weighed and sexed. Fetuses from all litters were examined externally for abnormalities. Litters from approximatley 20 females were were examined for skeletal and soft tissue abnormalities. Half of each litter was examined for skeletal abnormalities and the remaining half for soft tissue abnormalities.

Results

The report concludes; In conclusion, brodifacoum is neither teratogenic, embrotoxic nor fetotoxic in the rat at levels up to 0.02 mg/kg/day. It would appear unlikely that sublethal levels will effect embryonic or foetal development." Due to the lack of individual maternal observations and individual fetal data on the results of skeletal examination this conclusion could not be evaluated.

Discussion

The coumerin type anticoagulants, such as brodifacoum, act as vitamin K antagonists which inhibit the production of several clotting factors. Death occurs several days after a single lethal dose when the clotting factors in the blood have been sufficiently reduced by normal loss. Repeated doses also have a lag between first dose and maximum toxic effect (death). This is a combination of the necessity of 'building an effective dose' and the period of normal loss of clotting factors. This lag is particularly important in a teratology study if the period of maximum toxicity occured at the end of the period of fetal sensitivity rather during that period. Starting dosing at the beginning of the 'normal' exposure period can be expected to have produced such a delayed maximum effect in this study. Thus, in this study, the early fetus was not dosed to the maximum extent that was possible under the dosing regimen and we do not have a true test of the fetal toxicity of the compound.

Tox Chem No. Brodifacoum 114AA A File Last Updated ____ __ Current Date _____

Study/Lab/Study #/Date	Material	EPA MRID No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology-rabbit; ICI;CTL/P/459; 1/30/80	Tech 92.5%	52442	Doses tested, 0, 0.001, 0.002 &0.005 mg/kg/day; Lethal at 0.005 10/15 does deaths at the end of & after dosing period, no fetal effect. no individual animal/pup data. Cannot be corrected, dosing incorrect.	N/A	Unacceptable Report
Teratology-rabbit; ICI; CTL/P/459S; 8/13/87	Tech 92.5%	40372-01	Individual data to CTL/P/459 above incomplete no data on pup skeletons	N/A	Unacceptable Report
Teretology-rat; ICI;CTL/P/437; 1/22/80	Tech 92.5%	552443	Doses tested 0, 0.001, 0.01 & 0.02 mg/kg/day; no fetal effect, no individual animal/pup data. Cannot be corrected, dosing incorrect	N/A	Unacceptable report
Teratology-rar; ICI; CTL/P/437S;	Tech 92.5%	40372-02	Individual data to CTL/P/437 above incomplete no data on pup skeletons and maternal toxicity	N/A	Unacceptable Report