Appendix H:

The HED Chapter of the Reregistration Eligibility Decision Document (RED) for Rodenticide Cluster

B. Human Health Assessment

1. Toxicology Assessment

The toxicological data bases for, brodifacoum, bromadiolone, chlorophacinone, diphacinone and its sodium salt, and bromethalin are adequate and will support reregistration eligibility.

a. Acute Toxicity

(1) Brodifacoum Acute Toxicity

Results of the acute toxicity studies conducted with technical brodifacoum are summarized below in Table 5:

Table 5 - Acute Toxicity Values of Technical Brodifacoum

Route	Species	Results	MRID	Toxicity Category
Oral	Rat	LD_{50} (M) = 0.418 mg/kg LD_{50} (F) = 0.561 mg/kg	42687501	I
Dermal	Rabbit	LD_{50} (M) = 5.21 mg/kg LD_{50} (F) = 3.16 mg/kg	42232101	I
Inhalation	Rat	LC_{50} (M) = 4.86 µg/L LC_{50} (F) = 3.05 µg/L	43110501	I
Eye Irritation[a]	Rabbit	Some minor eye irritation, clearing by day 7.	66938	III
Skin Irritation[a]	Rabbit	Unlikely to cause anything more than mild irritation; the high toxicity (note the dermal LD_{50} values above) precludes necessity for testing the technical for dermal irritation potential.	None	-
Dermal Sensitization[a,b]	Guinea Pig	Non sensitizer	None	N/A

[a] Not required for TGAI, however, presented here for informational purposes.
[b] Conducted on the 0.25% Brodifacoum Formulation Concentrate; see below.

In an oral LD_{50} study in which technical brodifacoum (96.1%) was administered as a suspension in polyethylene glycol to 300 rats, there were no mortalities or signs of toxicity in males or females at 0.25 mg/kg, nor in males at 0.35 mg/kg (females were not tested at this dose level). However, 5/5 males and 1/5 females died following dosage at 0.5 mg/kg, and 5/5 females died following dosage at 0.75 mg/kg (males were not tested at this dose level). Signs of toxicity at 0.5 and 0.75 mg/kg included pallor, bleeding from the nose and/or rectum and/or other sites. Deaths occurred in the period from 3-8 days after dosing. *Post mortem* examination of those animals that died or were sacrificed *in extremis* and/or showed signs of bleeding, revealed the presence of free or clotted blood in the abdominal and/or thoracic cavity. Discoloration or pallor of a number of organs was also observed. These findings are consistent with the known anticoagulant activity of brodifacoum. The LD_{50} is calculated to be 0.418 mg/kg for males (95% confidence interval between 0.35 and 0.5 mg/kg) and 0.561 mg/kg for females (95% confidence interval 0.472-0.667 mg/kg). These results place brodifacoum in Toxicity Category I (MRID 42687501) by the oral exposure route.

In a dermal LD_{50} study with rabbits, brodifacoum technical (95.6%) was applied as a suspension in corn oil (500 mg/kg), olive oil (10 mg/kg), or polyethylene glycol 600 (1 mg/kg), with 24-hour occluded dermal exposure. At 500 mg/kg, all the males were euthanized *in extremis* on days 5-6, and all females between days 5 and 8. At 10 mg/kg, 4/5 males were found dead or were euthanized *in extremis* between days 7 and 11, and 5/5 females between days 6 and 8. The animals that died or were euthanized showed signs of extreme toxicity consistent with anticoagulant activity (pallor, bleeding/bruising, breathing abnormalities) immediately prior to death. There were practically no signs of skin irritation on any of the animals. The dermal LD_{50} of brodifacoum technical was calculated to be 5.21 mg/kg (95% confidence interval 1.95-13.8 mg/kg) for males, and 3.16 mg/kg (95% a.i. 1.00-10.00 mg/kg) for females. These results place technical brodifacoum in toxicity category I (MRID 42232101) in terms of dermal toxicity potential.

In an inhalation LC_{50} study in rats, groups of young adult Wistar-derived 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 or 4.40 µg/L. The mass median 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. Mortalities (accompanied by symptoms consistent with anticoagulant activity) occurred on days 4-6 in 3/5 males and 5/5 females exposed to the highest concentration (4.96 µg/L). The inhalation LC_{50} for males = 4.86 µg/L (based on particulate concentration), and for females = 3.05 µg/L. Brodifacoum technical (96.1%) is in toxicity category I (inhalation LC_{50} at or below 50 µg/L) based on the LC_{50} values in both sexes (MRID 43110501).

In an eye irritation study in rabbits, aliquots of 100 mg technical brodifacoum (92.5%) were instilled in the conjunctival sac of the left eye in each of 9 New Zealand white rabbits. Three of the rabbit eyes were irrigated for one minute with lukewarm tap water starting 30 seconds after instillation of the test material. In some of the rabbits, there was subsequent iritis and/or slight redness of the conjunctivae with slight chemosis and discharge; with all irritation clearing by day 7. Brodifacoum technical (92.5%) is in toxicity category III in terms of eye irritation potential (MRID 00066938). However, it is noted that because of the high toxicity of brodifacoum, absorption of any significant amount of the technical material by the ocular exposure route might result in mortality (and the animals in this study were followed for only 7 days after exposure). Technical brodifacoum is in toxicity category III in terms of its ocular irritation potential.

There are no dermal irritation studies on technical brodifacoum. Because of the relatively high toxicity, dermal exposure to undiluted (or mixtures containing a relatively high percentage of) technical brodifacoum would probably be fatal (the dermal LD_{50} of brodifacoum technical in rabbits is given above as 5.21 mg/kg for males, and 3.16 mg/kg for females).

Because of the high toxicity of technical brodifacoum, end-use products (mostly containing 0.005% brodifacoum) are usually manufactured from a formulation containing 0.25% brodifacoum. Results of the acute toxicity studies conducted with brodifacoum Formulation Concentrate are summarized below in Table 6:

Table 6 - Acute Toxicity Values of Brodifacoum Formulation Concentrate (0.25%)

Route	Species	Results	Toxicity Category	MRID
Oral	Rat	LD_{50} (M) = 163 mg/kg LD_{50} (F) = 152 mg/kg	II	44021701
Dermal	Rat[a]	LD_{50} (M) > 2000 mg/kg LD_{50} (F) > 2000 mg/kg	III	44021702
Skin Irritation	Rabbit	Test material stained the skin pink at application site, but no indication of an inflammatory response	IV	44021703
Dermal Sensitization	Guinea Pig	Evaluation complicated by pink staining at the application site, but no evidence of a sensitization response.	N/A	44021704

[a]Study conducted with rats; however, rabbits may be a more sensitive species

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

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

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

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

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

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

Brodifacoum Formulation Concentrate (0.25%) is in Toxicity Category III in terms of dermal toxicity potential, based on the dermal LD_{50} values in both sexes. It is noted that this study was conducted with rats as opposed to rabbits. Rats may be a less sensitive species than rabbits which are generally used in dermal toxicity studies.

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

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

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

In a dermal sensitization study (MRID 44021704) with brodifacoum Formulation Concentrate (0.25% a.i.), administered at challenge undiluted and as 30% and 10% w/v suspensions in deionized water, young adult Crl:(HA)BR male guinea pigs were tested using the method of Buehler. There were no indications of a sensitization reaction, although evaluation was complicated by pink staining at the application sites. Skin samples were examined histopathologically, with no indications of a significant inflammatory response. In this study, brodifacoum Formulation Concentrate (0.25% a.i.) is not a dermal sensitizer.

(2) Bromadiolone Acute Toxicity

The acute toxicity data for bromadiolone are summarized in Table 7.

Table 7 - Acute Toxicity Values for Bromadiolone

Study	Results	Category	MRID
Oral LD_{50}-rat[a]	between 0.56 and 0.84 mg/kg	I	41900001
Dermal LD_{50}-rabbit	1.71 mg/kg	I	42673701
Acute inhalation LC_{50}-rat	0.43 µg/kg	I	4197690
Eye irritation-rabbit	Irritation cleared by 4 days	III	88113
Dermal irritation-rabbit	Minimally irritating	IV	88112
Dermal sensitization	Not a dermal sensitizer	n/a	41847401

[a]This study was conducted with a concentrate which provides an understanding of the acute oral toxicity of bromadiolone.

A number of acute toxicity studies have been conducted with bromadiolone in the technical form or as a concentrate. The acute oral LD_{50} in rats was tested using a concentrate (2.5 gm/L) and doses were between 0.56 and 0.84 mg/kg (Toxicity Category I, MRID 41900001). An acceptable acute oral toxicity study with technical grade is currently unavailable, but the available data indicate that bromadiolone is very toxic. Requiring another acute oral toxicity with the technical grade may not add more information than what is currently available. The acute dermal LD_{50} in rabbits was 1.71 mg/kg (Toxicity Category I, MRID No. 42673701. This study satisfies Guideline 81-2 requirement). The LC_{50} for acute inhalation toxicity in rats is 0.43 µg/L (Toxicity Category I, MRID No. 41976901. This study satisfies Guideline 81-3).

A primary eye irritation study in rabbits indicated that bromadiolone technical produced no irritation in washed eyes. Conjunctivitis and iritis were seen in the unwashed eyes for 4 days. No corneal opacity was seen in either the washed or unwashed eyes (Toxicity Category III, MRID No. 00088113. This study satisfies the Guideline 81-4).

A primary dermal irritation study in rabbits showed that, after 24 hours of dermal application, bromadiolone produced minimal irritation on the application site (Toxicity Category IV; MRID No. 00088112. This study satisfies Guideline 81-5)

A dermal sensitization study in guinea pig showed that bromadiolone was not a dermal sensitizer (MRID No. 41847401. This study satisfies Guideline 81-6).

(3) Bromethalin Acute Toxicity

Results of the acute toxicity studies conducted with technical bromethalin are summarized below in Table 8:

Table 8 - Acute Toxicity Values of Technical Bromethalin

Route	Species	Results	Toxicity Category	MRID
Oral	Rat	LD_{50} (Males) = 10.7 mg/kg LD_{50} (Females) = 9.1 mg/kg	I	00026524
Dermal	Rabbit	LD_{50} = 2000 mg/kg	II	00026524
Inhalation	Rat	LC_{50} = 0.024 mg/L	I	00026524
Eye Irritation[a]	Rabbit	Slight irritation	III	00026524
Skin Irritation[a]	Rabbit	Not an irritant	IV	00026524
Dermal Sensitization[a]	Guinea Pig	Non sensitizer	N/A	41653001

[a]Not required for TGAI, however, presented here for informational purpose

An acute delayed neurotoxicity study was conducted in the hen. White rock strain hen (30 animals) were initially dosed with bromethalin in PEG-400 at 9 mg/kg and redosed on day 3 with 15 mg/kg. Observation was for 24 days. Bromethalin did not produce acute delayed neurotoxicity in the hen. (MRID 00101543).

An acute neurotoxicity study was conducted in rats. Male and female Sprague-Dawley CD rats were orally gavaged with bromethalin in mineral oil at doses of 0, 0.8, 1.5 or 3 mg/kg. The NOEL was greater than 3 mg/kg (HDT) and the LOEL was not determined in this study. Although this study was classified as unacceptable, the study can be upgraded if the registrant can provide the following data: the rationale of vehicle choice and volume used, the stability of test material in mineral oil, the rationale for choice of testing time on dosing day, and body temperature measurements. Body temperature is a measurement that should have been taken, given the mechanism of action of bromethalin (uncoupler of oxidative phosphorylation) (MRID 42793101). However, a new study will not be required since adequate information is available to determine an acute NOEL for bromethalin neurotoxicity.

(4) Chlorophacinone Acute Toxicity

Results of the acute toxicity studies conducted with technical chlorophacinone are summarized in Table 9:

Table 9 - Acute Toxicity Values of Technical Chlorophacinone

Route	Species	Results	Toxicity Category	MRID
Oral	Rat	LD_{50} (M) = 3.15 mg/kg LD_{50} (F) = 10.95 mg/kg combined = 6.26 mg/kg	I	41875301
Dermal	Rabbit	LD_{50} (M) = 0.329 mg/kg LD_{50} (F) = not done	I	41702801
Inhalation	Rat	LC_{50} (M) = 7 µg/L LC_{50} (F) = 12 µg/L	I	41981102
Eye Irritation[a]	Rabbit	No eye irritation at 1, 24, 48, or 72 hours.	IV	41874001
Skin Irritation[a]	Rabbit	PIS = 0, but mortalities occurred (same study as dermal LD_{50} assay)	IV	41702801
Dermal Sensitization[a,b]	Guinea Pig	Non sensitizer	N/A	41578601

[a] Not required for TGAI, however, presented here for informational purposes.
[b] 2/10 animals died

In an oral LD_{50} study in which technical chlorophacinone (99.36% by potentiometry, 102% by UV spectrophotometry) was administered as a suspension in polyethylene glycol 300 to Sprague-Dawley rats, there were mortalities at all dose levels in males (2.0 mg/kg: 4/10; 3.2 mg/kg: 6/10; 5.2 mg/kg: 4/10; 8.2 mg/kg: 8/10; 13.2 mg/kg: 10/10; 21 mg/kg: 9/10). There were no mortalities in females receiving doses of 2.0 or 3.2 mg/kg, but mortalities occurred at higher dose levels (5.2 mg/kg: 2/10; 8.2 mg/kg: 3/10; 13.2 mg/kg: 6/10; 21 mg/kg: 9/10). Deaths, with symptoms consistent with internal hemorrhage or other evidence of anticoagulant activity, occurred on days 4-13 after dosage. The acute oral LD_{50} for males was calculated as 3.15 mg/kg, with 95% confidence limits of 1.48-6.68 mg/kg. For females it was 10.95 mg/kg, with 95% confidence limits of 6.46-18.57 mg/kg. The combined oral LD_{50} for both sexes was calculated as 6.26 mg/kg (95% confidence limits of 3.96 to 9.89 mg/kg). These results place technical chlorophacinone in Toxicity Category I (MRID 41875301) by the oral exposure route.

In a dermal LD_{50} study with male New Zealand white rabbits chlorophacinone technical (100%) was dissolved in acetone and spread onto 2.0 x 2.0 cm pads. Each pad was allowed to dry before it was applied to a shaven dermal area on one of 10 male rabbits/dose level. Doses applied were 0.25, 0.5 or 0.75 mg/kg, with 24-hr occluded dermal exposure. Animals were observed for 21 days (instead of the usual 14 days) after exposure. Deaths occurred between days 5 and 19. Symptoms (which included bloody nasal discharge) and necropsy findings (hemorrhage in the thoracic cavity and large intestine) were consistent with anticoagulant activity. There were mortalities at each dose level (0.25 mg/kg: 4/10; 0.50 mg/kg: 6/10; 0.75 mg/kg: 9/10). There were no indications of skin irritation in any of the animals. The dermal LD_{50} of chlorophacinone technical was calculated to be 0.329 mg/kg (95% confidence interval 0.21-0.52 mg/kg) for males. Females were not tested. This was because males had been previously observed to be more sensitive to the anticoagulant effects of chlorophacinone than females. With a dermal LD_{50} below 200 mg/kg, technical chlorophacinone is in Toxicity Category I (MRID 41702801) by the dermal exposure route.

There were no indications of skin irritation from dermal exposure to technical chlorophacinone at doses which resulted in mortality (this is the dermal LD_{50} study indicated above, in MRID 41702801). The test material is in toxicity category IV in terms of its dermal irritation potential.

In an inhalation LC_{50} study in rats, groups of young adult Sprague-Dawley rats, 7-9/sex/exposure level, were exposed (nose only) for 4 hours to analytically-determined concentrations of 1.33, 10.3, 11.5 or 14.5 µg/L (the respective nominal values were 72.3, 88.63, 440 and 166 µg/L), with a subsequent 21-day observation. "To minimize human exposure, continuous observation of the animals during the 4-hour exposure was not maintained." Observations were made at 0.5, 1 and 2.5 hours during the exposure period. Between observations some animals turned in the restrainers and, as a result, died from suffocation. The deaths from suffocation were considered stress-related. All animals that died within the first 5 hours showed no clinical signs of hemorrhage. At the lowest concentration level (1.33 µg/L) there were no compound-related mortalities in 5 males and 7 females; but mortalities accompanied by signs of anticoagulant activity occurred on post-exposure days 3-8 in rats exposed to the higher concentrations (10.3 µg/L: 4/6 males, 2/8 females; 11.5 µg/L: 8/8 males, 5/6 females; 14.5 µg/L: 2/5 males and 3/6 females). The inhalation LC_{50} for males = 7.00 µg/L, with 95% confidence limits (C.L.) of 0.83 - 59 µg/L. For females, the inhalation LC_{50} = 12.0 µg/L, with 95% C.L. of 7.8 - 18 µg/L; and the combined LC_{50} = 9.3 µg/L, with 95% C.L. of 2.3 - 38 µg/L. Chlorophacinone technical (analyzed concentration: 101%) is in Toxicity Category I (inhalation LC_{50} at or below 50 µg/L) based on the LC_{50} values in both sexes (MRID 41981102).

In an eye irritation study in rabbits, 0.1 g technical chlorophacinone (99.88%) was instilled in the conjunctival sac of the left eye in each of 6 female New Zealand white rabbits, with no subsequent eye wash. Eyes were scored at 1, 24, 48 and 72 hours after exposure, but there were no indications of any irritation (all scores zero). Technical chlorophacinone (99.88%) is in Toxicity Category IV in terms of eye irritation potential (MRID 41874001). It is noted that the rabbits were only observed for 72 hours following ocular exposure, and the possibility exists that if observations had been continued mortalities might have subsequently been noted.

A dermal sensitization study (MRID 41578601) of male Hartley strain guinea pigs with chlorophacinone technical (99.88%), using the Buehler procedure and a 3-week induction period with 2 inductions/week was conducted. A first attempt was made using a dosage level of 0.2 g/animal/induction, but after one induction there was 40% mortality in the test group. In a second attempt, 0.01 g/animal/induction was used as a dose level. Subsequently, the dosage amount was reduced to 0.005 g/animal/induction using new animals. This part of the study was also terminated "due to high mortality in the test group." The final assay attempt utilized a dosage level of 0.003 g/animal/induction. Dosing chambers were secured with hypoallergenic tape, and following each 6-hour exposure period, the application site was wiped to remove as much of the test material as possible. Even so, two animals died during the induction period (on days 8 and 13). There were no indications of dermal irritation at the application sites during either the induction phase or following challenge. This study adequately demonstrates that technical chlorophacinone is not a dermal sensitizer as a result of exposure to non-lethal doses.

(5) Diphacinone and its sodium salt Acute Toxicity

Results of the acute toxicity studies conducted with technical diphacinone are summarized below in Table 10:

Table 10 - Acute Toxicity Values of Technical Diphacinone

Route	Species	Results	Toxicity Category	MRID
Oral	Rat	LD_{50} (M) = 2.5 (1.32-3.44) mg/kg LD_{50} (F) = 2.1 (1.55-2.86) mg/kg combined = 2.3 (1.86-2.88) mg/kg	I	00060605
Oral	Rat	LD_{50} (M) = 6.8 mg/kg LD_{50} (F) = 8.0 mg/kg combined = 7.0 (5.2-9.5 mg/kg	I	42245202
Dermal	Rabbit	LD_{50} (M) = 3.6 (0.6-20.8) mg/kg LD_{50} (F) = not done	I	42507001
Inhalation	Rat	LC_{50} (M) < 0.6 µg/L LC_{50} (F) < 0.6 µg/L	I	43000401
Eye Irritation	Rabbit	Moderate irritation clearing by day 4	III	42245203
Skin Irritation	Rabbit	Slight erythema clearing within 48 hours, but 4/6 rabbits died between days 8 and 10	IV	
Dermal Sensitization	Guinea Pig	neither a dermal irritant nor a sensitizer at a non-lethal dose level (2.5 mg/day)	N/A	42132501

In an oral LD_{50} study (MRID 00060605) technical diphacinone (purity not specified), was administered as a suspension in corn oil (volumes of 10 mL/kg were administered at all dosage levels) to Spartan rats (5/sex/dose level), at dose levels of 0, 0.79, 1.25, 1.98, 3.15, 5.00, 7.94, 12.60, 20.01, 31.76, 50.40 or 201.7 mg/kg, with a subsequent 14-day observation. The following mortality pattern was observed as outlined in Table 11.

Table 11 - Dose Levels and Mortality in an Oral LD_{50} Study with Diphacinone

Dose Level (mg/kg)	Males Deaths /Rats Dosed	Females Deaths /Rats Dosed	Combined Deaths /Rats Dosed	Days after dosage deaths occurred
0.79	0/5	0/5	0/10	-
1.25	0/5	1/5	1/10	6
1.98	2/5	1/5	3/10	4
3.15	3/5	5/5	8/10	3-7
5.00	5/5	5/5	10/10	3-6
7.94	5/5	5/5	10/10	3-7
12.60	5/5	5/5	10/10	4-6
20.01	5/5	4/5	9/10	3-8
31.76	5/5	5/5	10/10	4-9
50.40	5/5	5/5	10/10	3-7
201.70	5/5	5/5	10/10	3-6

Data extracted from tables 4, 5 and 6 of MRID 00060605

Symptoms occurred at all doses, and were not necessarily associated with subsequent mortality. These included clear or colored nasal discharge, soft stool and/or diarrhea (possibly associated with the corn oil vehicle used), decreased motor activity and occasional drying of the corneal surface. Symptoms at higher dose levels included lacrimation, ataxia, cyanosis and bloody exudate from nose and eyes. Hemorrhage into the body cavities and of various organs was observed in animals which died. The acute oral LD_{50} for males was calculated as 2.50 mg/kg, with 95% confidence limits of 1.82-3.44 mg/kg. For females. it was 2.10 mg/kg, with 95% confidence limits of 1.55-2.86 mg/kg. The combined oral LD_{50} for both sexes was calculated as 2.31 mg/kg (95% confidence limits of 1.86 to 2.88 mg/kg). These results place technical diphacinone in Toxicity Category I (MRID 00060605) by the oral exposure route. The study defines such a high degree of toxicity for technical diphacinone that the Agency can accept the findings, even in the absence of information as to the purity of the test material.

In a second oral LD_{50} study (MRID 42245202), technical diphacinone (reported as having "at least 98% purity") was administered as a 0.2% w/w suspension in corn oil to groups of 5 rats/sex/dose level. The dose levels were 4, 6, 8 or 10 mg diphacinone/kg body weight, with observation for 14 days after dosage. Signs of toxicity included nasal staining (usually red), paleness, red staining on the tail. Most animals that survived (including 2/3 at the highest dose level) appeared healthy throughout the test period. Necropsy findings of animals which died during the 14-day observation period were consistent with anticoagulant activity (such as red fluid in the thoracic and/or abdominal cavities, apparent testicular hemorrhage). The acute oral LD_{50} for males was calculated as 6.8 mg/kg, and for females 8 mg/kg. The combined oral LD_{50} for both sexes was calculated as 7 mg/kg (95% confidence limits of 5.2 to 9.5 mg/kg). The results of this second oral LD_{50} study (MRID 42245202) are reasonably consistent with those of the first (MRID 60605), as both define a Toxicity Category I hazard potential for technical diphacinone by the oral exposure route, although the second study indicates somewhat less toxicity (or perhaps the strain of rat used in the second study was less susceptible). See Table 12 below.

Table 12 - Dose Levels and Mortality in a Second Oral LD_{50} Study with Diphacinone

Dose Level (mg/kg)	Males Deaths/Rats Dosed	Females Deaths/Rats Dosed	Combined Deaths/Rats Dosed	Days after dosage deaths occurred
4	1/5	1/5	2/10	5
6	3/5	3/5	6/10	3-9
8	3/5	1/5	4/10	3-7
10	4/5	3/5	7/10	3-7

Data extracted from tables 1, 2, 5, 8 and 11 of MRID 42245202

It is noted that the mouse is considerably less susceptible to the toxic effects of diphacinone than other mammalian species (see discussion in Mutagenicity section under MRID 42406801). The NIOSH Registry of Toxic Effects of Chemical Substances 1985-86 reports a mouse LD_{50} for diphacinone as 300 mg/kg, and the rat LD_{50} as 1.5 mg/kg. This is also supported by a report from the open literature (Correll et. al., 1952) which states that the acute oral LD_{50} for diphacinone was found to be 3 mg/kg for rats, 340 mg/kg for mice, and 35 mg/kg for rabbits.

In a dermal LD_{50} study (MRID 42507001) with male New Zealand white rabbits, diphacinone technical (97.4%) was dissolved in acetone and the appropriate amount of the test substance solution was applied to the foil side of Scotch Pak pads. The acetone was allowed to evaporate, and the Scotch Pak pad, test substance side down, was applied to the application site, with 24-hour occluded exposure. In a range-finding trial, dose levels of 0, 1, 5, 10, 25 or 50 mg/kg were administered to groups consisting of 1 animal/sex/dose level. The findings in this range-finding study were used to set the doses in the subsequent definitive study. Females at the three highest dose levels - 10, 25, or 50 mg/kg - died. Those at the two lower dose levels - 1 and 5 mg/kg - survived. Deaths occurred on days 7-13. Males at all dose levels died. The performing laboratory suggested that only male rabbits should be used for the LD_{50} determination, as they had been the more sensitive sex. The doses for the dermal LD_{50} determination were 0.05, 0.20 and 0.80 mg/kg, with subsequent 21-day observation. The following mortality pattern was observed as outlined in Table 13.

Table 13 - Dose Levels and Mortality in a Dermal LD_{50} Study with Diphacinone

Dose Level (mg/kg)	Males Deaths/Rabbits Dosed	Days after dosage deaths occurred
0.05	0/10	-
0.20	1/10	4
0.80	2/10	4

Data extracted from tables IIA, IIB and IIC of MRID 42507001

The animals that died (both in the preliminary range-finding and subsequent LD_{50} determination studies), showed symptoms (hemorrhage, discoloration of various organs) indicative of anticoagulant activity. No clinical signs were observed at the lowest dose level (0.05 mg/kg). Symptoms at the two higher dose levels included somnolence, loss of fluids, absence of feces and vasoconstriction. Based on the mortality, the estimated dermal LD_{50} in male rabbits is 3.6 mg/kg. These results place technical diphacinone in Toxicity Category I (MRID 42507001) by the dermal exposure route.

In an inhalation LC_{50} study in rats (MRID 43000401), a group of young adult Sprague-Dawley rats, 5 of each sex, were exposed (whole body) for 4 hours to a time-weighted average aerosol concentration (gravimetrically determined) of 6 µg/L, with subsequent 14-day observation. The mass median aerodynamic diameter was 2.3 µm, with a geometric standard deviation of ± 2.1 µm. The percentage of particles ≤ 4.0 µm was equal to 78%. Mortality occurred (days 4-8) in 5/5 males and 4/5 females. Symptoms (including red staining of abdominal and urogenital regions, reddish material around ears and in cage tray) and necropsy findings (hemorrhage in the thoracic cavity and/or cranial cavity and/or various organs) were consistent with anticoagulant activity. The dose in the LC_{50} study was based on findings in a preliminary range-finding study, in which groups of one rat/sex/exposure level were exposed for one-hour to concentrations of 0, 0.01, 0.11, or 1.1 mg/L, with subsequent 7-day observation. All the males died (deaths occurred days 4-7), as did the female exposed to the lowest concentration (0.01 mg/L). However, the two females exposed to the two higher concentrations (0.11 and 1.1 mg/L) showed an array of symptoms (decreased activity, labored breathing, distended abdomen) on day 7 similar to those observed in other rats in the day or so before they died. These females underwent scheduled euthanasia. Diphacinone technical (percentage active ingredient not reported) is in Toxicity Category I (inhalation LC_{50} at or below 50 µg/L) based on the LC_{50} value of less than 6 µg/L in both sexes (MRID 43000401).

In an eye irritation study with New Zealand white rabbits (MRID 42245203), an attempt was made to place as much technical diphacinone (with at least 98% active ingredient) as possible into the conjunctival sac of one eye of each of nine rabbits. The report notes that the test material at 0.1 g exceeded the capacity of the rabbits' eye. The treated eyes of 3 rabbits were irrigated approximately 20-30 seconds after instillation of the test material. The eyes of the remaining 6 rabbits were not washed. No corneal opacity was observed, although some eyes showed iritis, and all eyes (washed and unwashed) showed some conjunctival irritation, with clearing by day 4. Technical diphacinone is in Toxicity Category III in terms of eye irritation potential (MRID 42245203). It is noted that the rabbits were only observed for 96 hours following ocular exposure, and the possibility exists that if observations had been continued mortalities might have subsequently been noted.

In a dermal irritation study with New Zealand white rabbits, 0.5 g of undiluted technical diphacinone (with at least 98% active ingredient) was applied to a single intact site, with 4-hour occluded exposure. Barely perceptible erythema was observed at 2 treated sites one hour after patch removal and at one treated site at 24 hours, with no evidence of erythema at 48 or 72 hours. No occurrence of edema was observed. The Primary Dermal Irritation Score was reported to be 0.09. Technical diphacinone (at least 98% active ingredient) is in Toxicity Category IV in terms of its primary dermal irritation potential. However, the report also notes "There were no signs of gross toxicity, adverse pharmacological effects or abnormal behavior during the test period. However, it should be noted that 4 of 6 rabbits died after the last scoring interval (i.e. between days 8 and 10 post-dosing). These spontaneous deaths may have been due to the anticoagulant properties of the test product."

In a dermal sensitization study (MRID 42132501) with Hartley albino male guinea pigs with diphacinone technical (96.57%), the test material was administered as a topical application

at various dose concentrations. The test article was kept in contact with the skin surface for a six hour period. After the initial exposure, the test article was administered on alternate days three days a week such that each animal received 10 sensitizing treatments. Following the tenth treatment, animals were rested for two weeks, and then given an eleventh (challenge) dose.

The major problem in this dermal sensitization study was on determining a non-lethal dose level. In the initial assay application of 500 mg caused death and/or severe hemorrhage from the external nares in some animals and evident discomfort in others, with the result that all surviving animals were euthanized. Further testing at doses of 5, 10, 20, 40 or 80 mg with two male guinea pigs/dose resulted in all animals either dying or being euthanized on or about the seventh day after the initial dose. Additional dosing at 0.1, 0.5, 1.0 or 2.5 mg with two animals/dose resulted in the death of one animal in the 0.5 mg group. As a result, the final dose selected was 2.5 mg in 10 guinea pigs (one of these animals died 13 days after the initial dose). Signs of dermal irritation were not observed in any of the guinea pigs at any dose level during the study, and there were no indications of any sensitization reaction in the survivors of the final assay (dose level: 2.5 mg/animal). There were 3 guinea pigs in a positive control group (each received 2.5 mg/application). One of these positive control animals died before the challenge application, but positive responses were elicited in the remaining 2 guinea pigs. The findings of this study (MRID 42132501) adequately demonstrate that technical diphacinone at a non-lethal exposure level is neither a dermal irritant nor a sensitizer.

b. Subchronic Toxicity

(1) Brodifacoum Subchronic Toxicity

The Agency has no record that any subchronic toxicity studies on brodifacoum have been received and/or reviewed. However, it is noted that there are a number of multiple-dose studies which the Agency has received (including a special study Brodifacoum: Blood Kinetics Study in the Pregnant Rat, MRID 42641902, see below), which include prothrombin time measurements, which appears to be the most sensitive indicator of toxicity for the anticoagulants.

Although the current toxicological data base is sufficient for the purposes of this RED, because of the potential for non-purposeful dermal exposure, and to more accurately assess the margins of exposure associated with potential incidental exposure, a 21-day dermal toxicity study (Guideline 82-2) is required as confirmatory data. Such a study must include prothrombin and activated partial thromboplastin time measurements, including pre-exposure, as well as on days 7, 14 and 21 of exposure.

(2) Bromadiolone Subchronic Toxicity

In a 90-day study, groups of beagle dogs (4/sex/dose) received bromadiolone in gelatin capsules at variable daily doses for different lengths of time. The dosages were low-dose, 5/10 µg/kg; mid-dose, 10/15/20 µg/kg; and high-dose, 15/25/50/100 µg/kg. The control dogs received starch in gelatin capsules. The high-dose animals died or were sacrificed moribund prior

to the study's termination. In addition, the high-dose animals also showed signs of loose, bloody stools following the 15 µg/kg dosing. After five days of following 100 µg/kg dosing high-dose animals also showed signs of hypothermia, respiratory difficulties, pale mucosa, drowsiness, atonia, bloody urine, hematomas, and external hemorrhage. Both mid- and high-dose dogs had increased prothrombin time and hematuria. Histological examination showed that in high-dose groups, 4/4 male or female dogs had hemorrhage, congestion and/or edema of the spleen, kidneys, lungs, urinary bladder, small intestine, liver, thyroid, and skin. No compound-related histological lesions were found in mid- and low-dose dogs. Based upon the clinical and hematological findings, the LOEL for subchronic toxicity of bromadiolone was 15 µg/kg; NOEL, 10 µg/kg (MRID 92196013).

In a multiple-dose toxicity study, groups of female rats (10/dose) received bromadiolone (technical grade) by gavage at doses of 6.4, 12.4, or 24.8 µg/kg for 20 days. By study day 13, the mid- and high-dose rats were all dead, and 8/10 rats in the 6.4 µg/kg group were also dead by day 20. The clinical signs included hemorrhage in the orbital sinus, nasal cavity, and nail beds, anorexia, and polydypsia. At necropsy, the dead rats showed general internal bleeding and hemorrhagic spots in liver, intestinal tract, and kidneys. No NOEL for subchronic toxicity could be established for bromadiolone (MRID 00107035).

The above two studies are classified as supplementary and do not meet the data requirements for a subchronic toxicity study in dogs and rats (Guideline No. 82-1). However, when the data from the 90-day dog study and the 20-day rat study are analyzed together with the results from rat and rabbit developmental toxicity studies, the results provided sufficient information for the understanding of the subchronic toxicity of bromadiolone. Additional subchronic toxicity tests would probably not yield much more new information. Therefore, a new subchronic toxicity study in either the rat or dog is not requested at this time.

(3) Bromethalin Subchronic Toxicity

Sprague Dawley rats (10/sex/group) received daily gavage doses of 0 (25% polyethylene glycol in H_2O), 5, 25, or 125 micrograms/kg/day (ug/kg/day) of bromethalin technical for 13 weeks. Parameters evaluated included daily observation, weekly body weight and food consumption, ophthalmoscopy, clinical pathology, necropsy, organ weights, and histopathology. The NOEL is 25 µg/kg/day. The LOEL is 125 µg/kg/day, based on spongy degeneration (leukoencephalomyelopathy) observed in most of the central white fiber tracts of the brain, cerebellum, pons, brain stem, and thoracic spinal cord of both sexes and optic nerves of males. There were no effects on mortality, clinical chemistry, ophthalmoscopy, body weight, food consumption, clinical pathology and histopathology of other tissues (MRID 43582102).

In a second 90-day study, groups of 4 male and 4 female beagle dogs were orally dosed by gavage for 90 days at levels of 0, 5, 25, 125, or 200 ug/kg/day with bromethalin technical. Observations included daily clinical evaluations, ophthalmoscopy, body weight, food consumption, clinical pathology evaluations at weeks 6 and 13, necropsy, organ weights and histopathology. The NOEL is 25 µg/kg/day. The LOEL is 125 µg/kg/day based on spongy

degeneration observed in nervous tissue components (cervical, thoracic, and lumbar spinal cord, brain stem, right and left optic nerves, frontal and median brain, pons, and cerebellum) in both sexes of dogs. At the high dose, 3 male dogs displayed the following neurotoxic signs before death or being sacrificed moribund: salivation and hypoactivity, followed by trembling, myoclonia, hyperesthesia, groaning, and decubitus. Other measured parameters were considered comparable between control and treated dogs of both sexes (MRID 43582101).

The above two subchronic toxicity studies in rats and beagle dogs are not guideline-type subchronic neurotoxicity studies. However, these studies will satisfy the data requirements for a 90-day neurotoxicity screening battery because a NOEL and a LOEL was established in both studies.

(4) Chlorophacinone Subchronic Toxicity

In a subchronic study (MRID 92018013), groups of 10 Sprague-Dawley rats/sex/dose were gavaged at 0, 10, 20 or 40 µg/kg 7 days/week for 113 days. A group was also dosed at 5 µg/kg/day, but was terminated at 77 days due to lack of evident toxicity. Additional groups were tested at 80 and 160 µg/kg, but all animals died between days 3 and 13. At 40 µg/kg/day deaths occurred in 10/10 males (mortalities occurred days 29-82) and 4/10 females (days 69-111); 4/10 males (but 0/10 females) died at 20 µg/kg/day (deaths occurred on days 105-111). "The dominant clinical signs that were responsible for death of animals were related to the anticoagulant activity of chlorophacinone." Although 1/10 males and 1/10 females died in the 10 µg/kg/day group, these deaths were ascribed to intubation error. At termination (112-113 days), hematology (including "coagulation time") and clinical chemistry parameters were determined from the 0, 10, 20 or 40 µg/kg/day groups (but not the 5 µg/kg/day group, which was terminated at 77 days). In the 10 µg/kg/day animals, males showed a 28% increase ($p < 0.01$) in coagulation time, while females showed a 6% increase ($p < 0.05$); at 20 µg/kg/day males showed a $> 100\%$ increase ($p < 0.01$) in coagulation time and females an 11% increase ($p < 0.05$); at 40 µg/kg/day females showed a $> 100\%$ increase.

The FIFRA 88 Phase 2 and 4 Data requirements for all anticoagulant rodenticides included a generic data request for a 14-day feeding study in the rat to determine a NOEL and LOEL for signs of toxicity and coagulation parameters. This information was requested to more adequately define and evaluate the effects that would result from accidental ingestion of this type of rodenticide. While MRID 92018013 does not adequately satisfy the Guideline requirements for a 90-day feeding or gavage study (Guideline 82-1), sufficient information is provided to satisfy the generic data request for a 14-day feeding study.

At the 5 µg/kg/day dose level there was no mortality or signs of toxicity during the 77-day exposure period. Coagulation values were not evaluated at this dose level. However clotting times were increased by 28% and 6% for males and females, respectively, at the 10 µg/kg/day levels at termination (113 days). Based on these findings, HED considers 5 µg/kg/day as a NOEL in a subchronic oral study, with a LOEL of 10 µg/kg/day (increased coagulation times for both males and females, with males more sensitive than females).

In a 21-day dermal toxicity study (MRID 42237402), a formulated product (tracking powder) containing 0.2% chlorophacinone was applied dermally with 6 hr occluded exposure/day, 5 days/week at 0.08, 0.40 or 2.0 mg/kg (these doses are in terms of the active ingredient, chlorophacinone) to 5 rabbits/sex/dose. The 0.2% product was used instead of the technical material because of difficulties (encountered in a preliminary range-finding study) in accurately weighing out and working with small quantities of this highly toxic compound. At 2 mg/kg/day, there was mortality (with "widespread" internal hemorrhage) in 4/5 males (deaths occurred on days 14-18) and 1/5 females (one death occurred on day 21). Prothrombin (PT) times were markedly increased on day 21 in surviving animals (the one male had a PT time of 9.0 seconds, while controls had a mean of 6.0. The females had a mean PT time of 17.7 seconds, as compared to a control mean of 5.9). Moderate to severe centrilobular liver necrosis was observed in 3/5 males and 1/5 females. There was no mortality at 0.4 mg/kg, but prothrombin times were markedly increased on day 21 (males: 7.7 vs. a control value of 6.0 seconds; females: 9.5 vs. a control value of 5.9). There were no indications of any effect at 0.08 mg/kg/day.

The following table from the report (in MRID 42237402) summarizes the measurements for prothrombin time (PT) and activated partial thromboplastin time in seconds (APTT):

Table 14 - Prothrombin and Activated Partial Thromboplastin Times in a 21-Day Subacute Dermal Study in Rabbits - Statistically Significant Findings

		Hematology Data - PT/APTT Mean Values							
Sex		Males				Females			
Parameter		Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
Dosage (mg/kg/day)		0	0.08	0.4	2.0	0	0.08	0.4	2.0
		Prothrombin time (PT) in seconds							
Pretreatment	Week -2	6.4	6.4	6.4	6.4	6.3	6.1	6.3	6.4
	Week -1	6.3	6.3	6.3	6.2	6.2	6.1	6.3	6.4
	Week - 0	6.6	6.3	6.3	6.3	6.4	6.3	6.2	6.5
Termination	Week - 0	6.0	6.0	7.7	9.0	5.9[a]	6.4[b]	9.5[c]	17.7[c]
		Activated partial thromboplastin time (APTT) in seconds							
	Week -3	32.5	32.4	52.3	24.5	22.9	28.3	59.7[c]	67.0[c]

[a]Examination of the female animals in the concurrent control, for the Week 3 interval, showed a statistically significant decrease based on their own three pretreatment values. This slight decrease in the control female value gave rise to the statistical significance in the Group 2 female value.

[b]Analysis of variance indicated a significant difference from the control value, $p \leq 0.05$; further statistical analyses, using repeated measures analysis of variance and dependent measures t-test procedures, indicated that this value did not vary significantly from the mean prothrombin time recorded at pretreatment intervals for those animals.

[c]Significantly increased, $p \leq 0.05$

The subchronic dermal LOEL is 0.4 mg/kg/day, based on increased prothrombin times in both sexes on day 21. The subchronic dermal NOEL is 0.08 mg/kg/day.

This subchronic dermal study in the rabbit is classified as acceptable (Guideline), and satisfies the guideline requirement for a subchronic dermal toxicity study (§82-2).

(5) Diphacinone and its sodium salt Subchronic Toxicity

In a 21-day dermal toxicity study (MRID 00074637), diphacinone (99.8%, moistened with 0.9% physiological saline) was applied (6-hr occluded exposure) five days a week for three weeks, at dosage levels of 0, 0.1, 1.0 or 10.0 mg/kg to groups consisting of four male and four female New Zealand white rabbits/dose level. The skin of two males and two females in each group was abraded. The skin of the remaining rabbits was left intact. Most of the animals exposed to diphacinone showed no dermal irritation. The dermal irritation which did occur in 1 or 2 animals/group was slight. However, all of the animals exhibited yellow staining of the test site after a few exposures.

Mortalities or sacrifice *in extremis* occurred in 1/8 controls, 1/8 in the 0.1 mg/kg/day group, 5/8 at 1.0 mg/kg/day, and 6/8 at 10.0 mg/kg/day. Symptoms included clear nasal discharge, pale skin and/or mucous membranes, and hypothermia. On gross pathology, "hemorrhagic areas in different sites were present in the stomach, mouth, ear, muscle, soft tissues, thoracic and abdominal cavities, cecum, colon, kidney and bladder of some animals. These lesions were more frequent in the 1.0 mg/kg and 10.0 mg/kg Diphacinone groups, only one case was present in the control group and one in the 0.1 mg/kg Diphacinone group." Blood samples were taken at preexposure, and on day 19. Determinations included hematocrit, hemoglobin, erythrocyte count, total leucocyte count, platelets, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. However, there were no measurements of clotting time.

Finally, while this 21-day dermal toxicity study (MRID 00074637) is classified as acceptable (satisfying the guideline requirement for a subchronic dermal toxicity study §82-2), with a subchronic dermal NOEL of 0.1 mg/kg/day, and a subchronic dermal LOEL of 1.0 mg/kg/day (based on mortality accompanied by indications of anticoagulant activity), it is noted that there are indications in the report of possible anticoagulant activity ("hemorrhagic areas") in one control and one 0.1 mg/kg rabbit. In addition, there were no clotting time determinations (such as prothrombin and/or activated partial thromboplastin times).

In a 21-day subchronic study (MRID 00077319), groups of 2 Swiss Webster mice/sex/dose level were intubated (using a 10 mg/mL solution of technical diphacinone in propylene glycol) at 0.1, 0.5, 1.0, 2.5, 5.0, 10.0 or 20.0 mg/kg/day for 20 days. All the mice dosed at 5, 10 or 20 mg/kg/day died during the first 7 days of the test period, and symptoms (bleeding, paleness) were generally consistent with anticoagulant activity. Three out of 4 intubated at 2.5 mg/kg died by day 14 (with symptoms of bleeding) with only one female surviving to termination. While there were no mortalities at 1.0 mg/kg, hemorrhages, sub-cutaneous accumulation of blood or external bleeding was noted at this dose level. No effects were observed at 0.5 mg/kg/day.

The observations in this study were subsequently used to set the dose levels (0, 0.1, 0.5, 1.0 or 2.5 mg/kg) in a mouse developmental toxicity study (also in MRID 00077319), which utilized 15 pregnant females/dose level. All animals dosed at 2.5 mg/kg/day died (days 4-10 of dosing). There was a considerable proportion of the fetuses in each female of this group

undergoing resorption (6/10, 4/10, 7/12, 3/11, and 4/12). At 1.0 mg/kg/day one pregnant female died on day 10 (2/10 fetuses were being resorbed).The LOEL in the 20-day feeding study is 1.0 mg/kg/day (occurrence of subcutaneous accumulation of blood, hemorrhages and external bleeding, with no mortalities), although no measurements were made for clotting time. While the single-dose LD_{50} for the mouse is about 300 mg/kg, the toxicity of diphacinone in this species is enhanced when administration takes place over a period of several days.

At the 0.5 mg/kg/day dose level there was no mortality or signs of toxicity during the 20-day exposure period, and no mortalities (or other effects) were observed at this dose level in the subsequently conducted mouse developmental toxicity study. The LOEL is 1.0 mg/kg/day (based on the occurrence of subcutaneous accumulation of blood, hemorrhages and external bleeding, with no mortalities in the initial 20-day study, and the occurrence of mortality in 1/5 pregnant females at this dose level in the subsequently conducted mouse developmental toxicity study). It is noted that no information is given in this study as to clotting times. While the information in MRID 00077319 is useful, it is not adequate to satisfy the FIFRA 88 Phase 2 and 4 data requirements for anticoagulant rodenticides for a 14-day feeding study in the rat to determine a NOEL and LOEL for signs of toxicity.

In a single dose toxicity study (MRID 43260702), male and female Sprague-Dawley rats (5/sex) received technical diphacinone (99.0%) as a single oral gavage dose in corn oil at doses of 0, 0.13, 0.20, 1.0 or 2.5 mg/kg. In a 14-day oral toxicity study (MRID 43260701) groups of 5 rats/sex/dose received technical diphacinone (99.0%) by oral gavage in corn oil once a day for 14 days at doses of 0, 0.025, 0.040, 0.085 or 0.175 mg/kg/day. The purpose of these experiments were to demonstrate a NOEL and LOEL for overt signs of toxicity, lethality, and anticoagulant effects in young adult Sprague-Dawley rats following single and repeated dosage with technical diphacinone. Following single doses at up to 2.5 mg/kg (HDT), there were no overt clinical signs of toxicity. Following repeated dosing, there were no signs of toxicity at the 0.025, 0.040 or 0.085 mg/kg/day dose levels. At the 0.175 mg/kg/day dose level, there were increased incidences of dyspnea, lethargy, hemorrhage from the nose, ptyalism, and few feces. At this dose level 3/5 males died (2/5 were found dead and one was sacrificed *in extremis*). All of the female rats in this dose group had died by day 11. The following Prothrombin (PT) and Activated Partial Thromboplastin Times (APTT) were observed as outlined in Tables 15, 16, 17 and 18.

Table 15 - Prothrombin Time in Seconds in Rats Following a Single Dose of Diphacinone[a]

	Prothrombin Time in Seconds				
Diphacinone (mg/kg)	0	0.13	0.20	1.00	2.50
Males - 24 hrs after dosing	15.5 ± 0.9	15.1 ± 0.5	15.2 ± 0.2	56.6 ± 9.2	70.7 ± 8.1
Males - 96 hrs after dosing	14.0 ± 0.3	14.5 ± 0.3	14.3 ± 0.5	14.3 ± 0.3	22.9 ± 16.6
Females - 24 hrs after dosing	15.1 ± 0.3	14.8 ± 0.4	15.6 ± 0.5	30.9 ± 6.6	51.8 ± 14.9
Females - 96 hrs after dosing	14.4 ± 0.2	14.6 ± 0.4	14.4 ± 0.3	14.0 ± 0.3	15.1 ± 0.9

[a]Data taken from pages 51-58 of the report (MRID 43260702)

Table 16 - Activated Partial Thromboplastin Time in Seconds in Rats Following a Single Dose of Diphacinone[a]

	Activated Partial Prothrombin Time in Seconds				
Diphacinone (mg/kg)	0	0.13	0.20	1.00	2.50
Males - 24 hrs after dosing	24.5 ± 2.6	22.1 ± 3.5	24.0 ± 1.3	49.8 ± 18.3	42.2 ± 5.3
Males - 96 hrs after dosing	21.7 ± 3.1	21.2 ± 2.7	19.4 ± 2.8	21.1 ± 1.7	30.3 ± 11.0
Females - 24 hrs after dosing	20.3 ± 1.6	19.3 ± 1.1	32.1 ± 6.6	39.6 ± 3.6	33.9 ± 7.8
Females - 96 hrs after dosing	21.4 ± 3.9	19.8 ± 2.0	18.4 ± 0.5	20.6 ± 1.7	29.3 ± 4.5

[a]Data taken from pages 51-58 of the report (MRID 43260702)

Table 17 - Prothrombin Time in Seconds in Rats Following Repeated Doses of Diphacinone[a]

	Prothrombin Time in Seconds				
Diphacinone (mg/kg/day)	0	0.025	0.040	0.085	0.175
Males - 24 hrs after last dose	14.0 ± 0.2	14.2 ± 0.2	14.4 ± 0.2	15.2 ± 0.7	20.0 ± 1.3
Males - 96 hrs after last dose	14.4 ± 0.4	14.4 ± 0.3	14.0 ± 0.7	14.1 ± 0.4	14.3 ± 0.4
Females - 24 hrs after last dose	14.3 ± 0.3	14.6 ± 0.3	14.4 ± 0.2	14.4 ± 0.3	--b
Females - 96 hrs after last dose	14.4 ± 0.8	14.6 ± 0.3	14.3 ± 0.4	14.7 ± 0.1	--b

[a]Data taken from pages 51-58 of the report (MRID 43260702)
[b]All animals were dead by day 11

Table 18 - Activated Partial Thromboplastin Time in Seconds in Rats Following Repeated Doses of Diphacinone[a]

	Activated Partial Thromboplastin Time in Seconds				
Diphacinone (mg/kg/day)	0	0.025	0.040	0.085	0.175
Males - 24 hrs after last dose	20.2 ± 1.1	20.0 ± 1.2	22.0 ± 1.2	25.9 ± 2.1	38.3 ± 11.0
Males - 96 hrs after last dose	20.4 ± 2.0	20.3 ± 1.0	19.9 ± 1.0	19.9 ± 0.9	19.7 ± 0.7
Females - 24 hrs after last dose	20.7 ± 1.9	19.7 ± 0.8	20.6 ± 0.7	24.4 ± 2.1	--b
Females - 96 hrs after last dose	21.4 ± 2.9	20.2 ± 0.9	20.2 ± 0.5	20.0 ± 0.9	--b

[a]Data taken from pages 51-58 of the report (MRID 43260702)
[b]All animals were dead by day 11

The LOEL from single dose administration is 0.20 mg/kg, based on increased activated partial thromboplastin time in female rats. The NOEL from single dose administration is 0.13 mg/kg. The LOEL from repeated dose administration is 0.085 mg/kg/day, based on increased prothrombin and activated partial thromboplastin times in male and female rats. The NOEL from repeated dose administration is 0.040 mg/kg/day. The information in MRIDs 43260701 and 43260702 satisfies the FIFRA 88 Phase 4 Data requirements to determine effects in the rat (and defining the NOELs and LOELs for signs of toxicity and coagulation parameters) following a single dose and following repeated oral dosage over a 14-day period.

c. Chronic toxicity

Given the exclusively non-food uses of these chemicals, no chronic studies were required.

d. Carcinogenicity

Given the exclusively non-food uses of these chemicals, no carcinogenicity studies were required.

e. Developmental Toxicity

(1) Brodifacoum Developmental Toxicity

In a developmental toxicity study (MRID 00052443, along with additional data in MRID 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 or 0.02 mg/kg/day from days 6 through 15 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, see below).

The rat maternal toxicity 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 rat 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 in the rat.

In a special study (MRID 42641902), mixtures of unlabeled brodifacoum (98.7%) and radiolabeled 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) or 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 nanogram (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.

This study showed 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 that study. three rats were given a single oral dose of 0.25 mg labeled brodifacoum and 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 (Non-guideline) 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).

In a developmental toxicity study in rabbits (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 or 0.005 mg brodifacoum/kg/day from days 6 through 18 of gestation. Ten of the 15 rabbits receiving 0.005 mg/kg/day died or were humanely euthanized. 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; euthanized 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 was not reported for the 0.005 mg/kg/day females. 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 following table shows the prothrombin time measurements (presumably in seconds) on day 20 in a preliminary range-finding study.

Table 19 - 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)

The rabbit 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). The developmental toxicity NOEL is 0.002 mg/kg/day, as only 3 litters (with a total of 20 fetuses) were available for evaluation at 0.005 mg/kg/day). It is reported that all of the implants from a 0.005 mg/kg/day doe which was euthanized on day 16 had a hemorrhagic appearance. This developmental toxicity study in rabbits is classified as acceptable (Guideline 83-3b).

(2) Bromadiolone Developmental Toxicity

Groups of pregnant Sprague-Dawley rats received bromadiolone (technical grade) in aqueous vehicle by gavage from gestation days (gd) 6 through 16 at doses of 0, 17.5, 35, and 70 µg/kg bw/day. There was an increase in the incidence of vaginal bleeding, hypotonicity, pale eyes, and deaths in 70 µg/kg dams. None of the above findings were seen in the controls or the two lower dose groups. No developmental toxicity was found in the test animals. The NOEL for developmental toxicity was 70 µg/kg (HDT). Based on the increased incidence of vaginal bleeding, hypotonicity, pale eyes, and deaths, the LOEL for maternal toxicity was 70 µg/kg. The NOEL was 35 µg/kg. This study satisfies the data requirements for a developmental toxicity study in rats (Guideline No. 83-3(a); MRID No. 92196014).

Groups of artificially inseminated New Zealand White rabbits received bromadiolone (99.8% purity) in aqueous media by gavage from gestation days (gd) 6 through 18 at doses of 0, 2, 4, and 8 µg/kg bw/day. Vaginal bleeding was found in 8/19 does of the 8 µg/kg group, in 1/19 does of the 2 µg/kg group, and none in the 4 µg/kg group and the controls. Since bromadiolone is an anticoagulant, the vaginal bleeding seen in the 2 µg/kg group could be conservatively considered as a compound-related effect in spite of the lack of a dose-related response. The prothrombin times of the highest dose group and the controls were comparable at sacrifice (11 days after dosing). This result was consistent with that seen in an antidote study where bromadiolone (up to 5.6 mg/kg bw) did not affect the prothrombin times of rats which received bromadiolone in the diet 2 weeks prior to the prothrombin time measurement (Tox. Document No. 009423; MRID No. 420933-01). Under the conditions of this study, conservatively, the incidence of vaginal bleeding seen in the lowest dose group (2 µg/kg) was considered as a threshold effect. The Peer Review/RfD Committee had analyzed the results of this study, and considered the 2 µg/kg as the "threshold" NOEL. The LEL was 4 µg/kg. There was no developmental toxicity in any dose group, and the NOEL for developmental effect was 8 µg/kg (HDT). This study satisfies the data requirements for a developmental toxicity study in rabbits (Guideline No. 83-3(b); MRID No. 92196015).

(3) Bromethalin Developmental Toxicity

A developmental toxicity study was conducted with Harlan Wistar rats (25 rats/group). Rats were orally gavaged on gestation days 6 through 15 at a dosing volume of 5 ml/kg with 0 (vehicle, PEG-200), 0.1, 0.3, or 0.5 mg/kg/day bromethalin technical. Surviving dams were sacrificed on gestation day 20, necropsied and reproductive findings were recorded. The NOEL for developmental toxicity is 0.5 mg/kg/day (HDT). There were no compound-related external, visceral or skeletal effects in bromethalin-treated fetuses in comparison to controls on either a litter or fetal basis.

The NOEL for maternal toxicity is 0.3 mg/kg/day and the LOEL is 0.5 mg/kg/day. Several effects occurred at the 0.5 mg/kg/day including four deaths during gestation (gestation days 12, 16, 17, and 17). Three high-dose females revealed upper respiratory tract infections which was regarded as secondary due to physiological stress from treatment. Additionally, in 10 of the 25 high-dose females, including the four which died, clinical signs consisting of hind leg weakness and decreased muscle tone were seen. Other observations included poor grooming, weakness, ventral soiling, chromodacryorrhea, decreased respiration, labored respiration, hypothermia, hind leg paralysis, prostration and dehydration.

During the dosing period, high-dose dams had a 30.2% decrease in weight gain in comparison to controls. During the post dosing period, weight gain in the high-dose females was decreased by only 11.7% in comparison to controls. Due to the substantial decreased weight gain during the dosing period, the high-dose females experienced a 13.9% decrease in weight gain for the entire gestation period in comparison to controls. These decreased weight gains are considered to be treatment-related. Food consumption was decreased by 8.7% in high-dose animals in the post dosing period in comparison to controls. The observed decrease in weight gain during the post dosing period may be because of decreased food consumption. The food consumption was comparable between controls and treated groups, including the high-dose group at other times (MRID 00086731).

A second developmental toxicity study was conducted with Dutch Belted rabbits (15/group). In this study, rabbits were orally gavaged at a volume of 1 ml/kg with bromethalin at doses of 0 (PEG-200, vehicle), 0.10, 0.25, or 0.50 mg/kg/day during gestation days 6 through 18. Surviving does were sacrificed on gestation day 28 and reproductive parameters were determined. The NOEL for developmental toxicity is 0.5 mg/kg/day (HDT). There were no compound-related external, visceral or skeletal effects in bromethalin-treated fetuses in comparison to controls on either a litter or fetal basis.

The NOEL for maternal toxicity is 0.10 mg/kg/day. Clinical signs of toxicity were observed in two females at 0.25 mg/kg/day and 5 females in the 0.50 mg/kg/day group. These signs included nasal discharge, loss of muscle tone, weakness, decreased respiration, coolness, and prostration. Two high-dose does died; one on gestation day 16 and one on day 21. The two high-dose does that died had clinical signs before death. One female that died had pneumonia and an empty gastrointestinal tract, and the other had an acute upper respiratory tract infection. Additionally, two high-dose does, one mid-dose doe and one low-dose doe aborted. The two high-dose and the one low-dose does that aborted had gastric trichobezoars in an otherwise empty gastrointestinal tract. The mid-dose doe which aborted had an empty gastrointestinal tract. The clinical signs, abortions and deaths at the top dose and the clinical signs at the mid-dose are considered compound-related. Mid and high-dose animals had decreased weight gains during the dosing period, which are considered compound-related. Food consumption was comparable between control and treated does during gestation. Although values for the mid-dose animals were lower than controls, this finding was not dose-related and is not considered compound-related (MRID 00101545).

(4) Chlorophacinone Developmental Toxicity

In a preliminary range-finding study in rats (MRID 43349501) chlorophacinone (analytically determined concentration 101%) was administered at days 6-15 of gestation at doses

of 0, 1, 5, 25, 50, 100 or 200 µg/kg/day to groups of 8 mated Sprague-Dawley female rats. Mortalities occurred at 100 and 200 µg/kg/day. Five rats/dose level in the 0, 1, 5, 25 and 50 µg/kg/day groups were sacrificed on gestation day 16, and prothrombin and activated partial thromboplastin times were determined (Refer to Table 20).

It is noteworthy that while there was clotting in at least one sample from the controls and 3 lowest dose groups, this apparently did not occur in the 5 samples from rats of the 50 µg/kg/day group.

Table 20 - Prothrombin (PT) and Activated Partial Thromboplastin Times (APTT) in a Preliminary Rat Developmental Toxicity Study

	Dose Level (µg/kg/day)				
	0	1	5	25	50
Prothrombin Time (sec)[a]	12.2 ± 0.6 N = 4[b]	12.9 ± 1.3 N = 2[b]	12.8 ± 0.3 N = 4[b]	12.6 ± 0.4 N = 3[b]	13.0 ± 0.2 N = 5
Activated Partial Thromboplastin Time (sec)[a]	15.5 ± 2.1	23.9 ± 12.4	16.1 ± 1.4	16.2 ± 1.3	17.0 ± 0.9

[a]Reported as the mean ± S.E.M.

[b]decrease in N is due to the clotting of some of the samples on which the analysis could not be done.

In the subsequent developmental toxicity study (also in MRID 43349501) chlorophacinone (analytically determined concentration: 101% a.i.) was administered to groups of 25 Sprague-Dawley female rats/dose level by gavage at doses of 0 (vehicle only), 12.5, 25, 50 or 100 µg/kg/day on gestation days 6-15 inclusive. The test compound was administered as a suspension in corn oil. Eighteen high-dose (100 µg/kg/day) rats died or were sacrificed moribund (gestation days 12-16) with necropsy findings (blood in vagina and amniotic sacs, blood in stomach and/or small and/or large intestines) indicative of anticoagulant effects. There were no indications of maternal toxicity at 50 µg/kg/day. Treatment-related effects for developmental anomalies, were noted at the lowest dose and above as increased fetal and litter incidences of distended ureter (Refer to Table 21).

Table 21 - Fetal and Litter Incidences of Treatment Related Effects in a Rat Developmental Toxicity Study (doses in µg/kg/day)

	Control 0	Low 12.5	Low Mid 25	High Mid 50	High 100
# pups/# litters examined	205/25	186/24	206/25	196/24	55/7
Hydroureter:					
Bilateral	4/4	8/4	23/10	21/9	12/3
Left	2/2	3/3	3/3	5/4	0/0
Right	0/0	0/0	0/0	1/1	1/1
TOTAL INCIDENCE	6/6	11/5	26/11	27/11	13/4
% Incidence	2.9/24.0	5.9/20.8	12.6/44.0	13.8/40.7	23.6/57.1
Distended ureter:					
Bilateral	1/1	2/2	3/2	4/4	1/1
Left	1/1	4/3	3/3	6/6	1/1
TOTAL INCIDENCE	2/2	6/4	6/5	10/7	2/2
% Incidence	1.0/8.0	3.2/16.7	2.9/20.0	5.1/25.9	3.6/28.6
Total ureter anomaly:					
incidence:	8/6	17/10	32/13	37/14	15/5
% Incidence	3.9/24.0	9.1/41.7	15.5/52.0	18.9/51.9	27.3/71.4

At the highest dose (100 µg/kg/day) there was an increased total incidence (16/55 fetuses in 5/7 litters; controls: 14/205 fetuses in 10/25 litters) of enlarged lateral ventricle. At 50 µg/kg/day there was an increased incidence of extra rib on lumbar vertebrae I (not noted at 100 µg/kg/day; however, fewer litters were available for examination). For malformations, there were increased fetal and litter incidences of bilateral hydroureter at 25 µg/kg/day.

The rat maternal toxicity NOEL = 50 µg/kg/day.
The rat maternal toxicity LOEL = 100 µg/kg/day (based on mortality)
The rat developmental NOEL is < 12.5 µg/kg/day.
The rat developmental LOEL is < = 12.5 µg/kg/day (increased incidences of hydroureter, distended ureter and total ureter anomaly).

This developmental toxicity study in the rat is classified as acceptable and satisfies the guideline 83-3(a) requirement for a developmental toxicity study in the rat.

In a preliminary range-finding developmental toxicity study in rabbits (MRID 43570801). chlorophacinone (analytically determined concentration 101%) was administered at 0, 1, 2, 5, 10, 50 or 100 µg/kg/day to groups of 5 mated female rabbits. In addition, there were five satellite groups, each containing 3 rabbits dosed at 0, 1, 2, 5 or 10 µg/kg/day. The dosing period was from gestation days 7 through 19; satellite females were sacrificed on gestation day 20 and their blood was analyzed for Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) measurements. Both the mean PT and APTT were elevated in the 10 µg/kg/day females (refer to Table 22).

Table 22 - Prothrombin (PT) and Activated Partial Thromboplastin Times (APTT) in a Preliminary Rabbit Developmental Toxicity Study Chlorophacinone (µg/kg/day)

	0	1	2	5	10
No. of female rabbits bled	3	3	3	3	3
Prothrombin Time (sec)[a]	8.1 ± 0.5	7.8 ± 0.2	7.9 ± 0.1	8.7 ± 0.6	11.6 ± 2.1
Activated Partial thromboplastin Time (sec)[a]	26.5 ± 5.7*	26.6 ± 3.7	23.2 ± 1.5	26.4 ± 4.9	53.0 ± 14.3

[a]Reported as the mean ± S.E.M.
*p < 0.05; Jonckheere's Test (significant by trend test)
Table from page 167 of MRID 43570801.

In the subsequent developmental toxicity study in rabbits (MRID 43570801), chlorophacinone (analytically determined concentration reported as 101%) was administered to 16 New Zealand white rabbits/dose level by oral gavage at dose levels of 0, 5, 10, 25 or 75 µg/kg/day from gestation days 7 through 19, inclusive.

There was maternal mortality in 13/16 high mid (25 µg/kg/day) and 16/16 high dose (75 µg/kg/day) rabbits, with hemorrhage (neck, thoracic cavity, vagina, uterus, amniotic sacs, and GI tract). Increased incidences of external bleeding around the mouth, ears, and urogenital system, along with pale eyes, ears, lips/gums, lethargy and blood in the pan beneath the cage, were noted in the two highest dose groups. No evidence of treatment-related fetotoxicity was

noted in the cesarean section observations. However, due to the low number of surviving litters (3) at 25 µg/kg/day, and the lack of surviving litters at the highest dose (75 µg/kg/day), developmental toxicity cannot be assessed at these doses, and 10 µg/kg/day will be considered as the NOEL for developmental toxicity. This developmental toxicity study in the rabbit is classified as Acceptable (Guideline 83-3(b), and satisfies the guideline requirement for a developmental toxicity study in the rabbit.

The rabbit maternal toxicity NOEL is 5 µg chlorophacinone/kg/day. The LOEL is 10 µg/kg/day (based on increased prothrombin and activated partial thromboplastin times in the preliminary range-finding study. These measurements were not made in the subsequent developmental toxicity study). The rabbit developmental toxicity NOEL is 10 µg/kg/day, based on the lack of sufficient fetuses/litters at the next highest dose level (25 µg/kg/day) available for evaluation. This developmental toxicity study (Guideline 83-3(b) in the rabbit is classified as acceptable.

(5) Diphacinone and its sodium salt Developmental Toxicity

In a developmental toxicity study in rats (MRID 42834801), technical diphacinone (purity > 97%) in corn oil was administered via gavage to groups of 25 mated female Sprague-Dawley rats/dose level at 0, 10, 25 or 75 µg/kg/day on gestational days 6-15, inclusive. There were no effects on maternal body weight or weight gain. Reddish vaginal discharge, reddish urogenital staining and/or reddish fluid in the cage/tray were observed in one dam from the control group, two dams at 10 µg/kg/day, three dams at 25 µg/kg/day, and six dams at 75 µg/kg/day. One dam (with these symptoms) in the 75 µg/kg/day group was euthanized *in extremis* on day 15. A NOEL was not established for maternal toxicity then, as there was a dose-related increase in incidence of clinical signs of the anticoagulant effects of diphacinone through all dose levels. No compound-related altered growth and/or developmental anomalies were observed. There was an increased number of early resorptions and resorptions/dam at 75 µg/kg/day (52 and 2.2 ± 1.8, respectively, compared to control values of 33 and 1.4 ± 1.5). These increases were not statistically significant, and were within the upper limit of historical control data. However, 33% mortality was observed at 100 µg/kg/day in a range-finding study, and the increased number of resorptions is consistent with what was observed in a mouse developmental toxicity study (MRID 00077319) at a dose level (2.5 mg/kg/day) at which 5/5 pregnant females died.

The rat maternal toxicity NOEL < 10 µg/kg/day.
The rat maternal toxicity LOEL = 10 µg/kg/day (based on signs consistent with anticoagulant activity)
The rat developmental NOEL = 25 µg/kg/day.
The rat developmental LOEL = 75 µg/kg/day (based on an increased incidence of resorptions)

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

It is noted that no clotting time measurements were obtained in this rat developmental toxicity study, and that there is no rabbit developmental toxicity study. The Agency has received, for brodifacoum and chlorophacinone, both rat and rabbit developmental toxicity studies. For both of these anticoagulants, the rabbit is the more sensitive species, particularly with respect to mortality.

f. Mutagenicity

Results of mutagenicity studies for brodifacoum, bromadiolone, bromethalin, chlorophacinone and diphacinone and its sodium salt indicate the following:

Salmonella typhimurium. There were no indications of an increased number of revertants at the histidine locus in any of the strains used.

In Vivo Testing. While different species were used such as Chinese hamsters and mice, results were consistent and there was no evidence of induced mutagenicity response to any strains at any non-activated or activated dose levels.

In Vitro Testing. Testing was performed for chlorophacinone and bromadiolone. Based on this testing it can be concluded that at doses up to and including those associated with cytotoxicity (50 µg/ml), did not induce a clastogenic response in human lymphocytes under the conditions of this assay either in the presence or absence S9.

Appendix C of this document provides the MRID numbers and names of studies used to support these mutagenicity findings.

g. Metabolism

(1) Brodifacoum Metabolism

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

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

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

Although only one metabolite (the glucuronide) is identified, it is the parent compound which is of toxicological concern, and the registrant has adequately demonstrated in previously submitted studies (refer to MRIDs 00080235 and 42007502) that a high proportion of unmetabolized compound is retained, particularly in the liver.

In a second study (*in vitro* perfusion, also in MRID 44021705) the lower vena cava of a single male rat was ligated. The hepatic portal vein was then cannulated and the liver was cleared of blood and the bile duct cannulated. The liver was perfused and, after equilibration, ^{14}C-brodifacoum, at a dose of 10 mg/kg, was added to the main perfusate reservoir. Bile and perfusate were collected at pre-dose, 1 minute (perfusate only), 1, 2, 3, 4 and 6 hr post-dose. The radioactivity present in bile, perfusate, terminal perfusate supernatant, supernatant filtrate and liver was determined. There was 74.32% recovery after 6 hours, with 59% of the total in perfusate, and 15.19% in liver. Metabolite profiling was attempted, but no metabolites were identified. All radioactivity in the perfusate supernatant was bound to perfusate proteins, with no activity being measured in the aqueous filtrate.

In a metabolism study (MRID 42007502), groups of male rats received single oral doses of ^{14}C-labeled brodifacoum at different dose levels (Group 2: 0.02 mg/kg; Group 3: 0.15 mg/kg; Group 4: 0.35 mg/kg), and blood was taken from 1-3 rats/group at various intervals following this dosage. The following Kaolin Cephalin Time (KCT) and Prothrombin Time (PT) measurements were made as outlined in Table 23 below:

Table 23 - Kaolin Cephalin and Prothrombin Time in a Metabolism Study in Male Rats

	Clotting times (seconds)					
	Group 2: 0.02 mg/kg		Group 3: 0.15 mg/kg		Group 4: 0.35 mg/kg	
Time after Dosing	KCT	PT	KCT	PT	KCT	PT
6 hr	-	-	-	-	ND	14.3 ± 1.7
12 hr	-	-	-	-	ND	20.7 ± 3.7
18 hr	-	-	-	-	43.7 ± 2.1	37.2 ± 5.4
24 hr	14.9[a] ± 4.2	13.0[a] ± 1.8	15.8 ± 4.8	13.0 ± 1.1	58.9 ± 7.6	95.5 ± 2.7
48 hr	-	-	-	-	113.7 ± 10.6	147.6 ± 6.9
72 hr	-	-	-	-	92.8 ± 49.4	39.7 ± 19.4
96 hr	-	-	-	-	32.3 ± 7.2	18.8 ± 2.0
Day 8	-	-	-	-	21.3[a] ± 2.4	15.8[a] ± 1.2
Day 14	-	-	14.0 ± 1.1	14.3 ± 0.2	15.4 ± 4.5	17.4 ± 0.5
Day 28	14.9[a] ± 1.1	12.7[a] ± 0.3	21.3 ± 2.9	13.6 ± 0.6	20.2 ± 2.9	13.4 ± 0.4
Day 56	-	-	16.2[a] ± 2.4	12.7[a] ± 0.6	19.6[a] ± 2.2	13.3[a] ± 0.2
Day 84	-	-	-	-	17.2 ± 2.9	12.5 ± 0.4
Week 13	14.1[a] ± 1.1	15.4[a] ± 0.6	16.5 ± 1.4	13.8 ± 0.2	-	-
Week 26	-	-	12.3[b]	16.1[b]	-	-
Week 39	16.6 ± 4.3	13.5 ± 1.2	15.0 ± 1.7	13.8 ± 0.5	-	-
Week 52	-	-	15.6 ± 6.2	12.7 ± 1.2	-	-
Week 65	16.7 ± 3.3	13.5 ± 0.8	18.0 ± 3.2	13.2 ± 0.5	-	-
Week 78	-	-	18.6 ± 1.3	12.8 ± 1.2	-	-
Week 91	16.8 ± 2.0	14.6 ± 0.4	19.8 ± 2.2	15.1 ± 1.5	-	-
Week 104	14.7 ± 3.0	11.1 ± 1.0	13.2 ± 0.5	10.9 ± 0.6	-	-

The standard deviation (SD) is derived from data obtained with 3 animals per group.
[a] 2 values only
[b] single value only
ND = not determined
Table taken from p. 26 of MRID 42007502.

The results given above clearly show an increase in clotting time in rats which had received a single oral dose of 0.35 mg/kg. Assuming the effect was manifested as a doubling of the normal clotting time (to approximately 30 seconds for kaolin cephalin and/or prothrombin times), effects were evident as soon as 18 hours after dosage, and were still present at 96 hours post-dosage. In addition, the metabolism study in MRID 42007502 demonstrates that considerable amounts of the radiolabel are retained in the liver following dosage (refer to the Table 24).

Table 24 - Percentage of radioactivity retained in the liver following single-dose administration of ^{14}C Brodifacoum

Time after dosing	Group 2: 0.02 mg/kg Mean SD	Group 3: 0.15 mg/kg Mean SD	Group 4: 0.35 mg/kg Mean SD
Day 1	47.33 ± 10.87	29.71 ± 4.40	28.92 ± 1.79
Week 4	39.16 ± 3.50	37.07 ± 1.94	23.47 ± 1.21
Week 8	-	30.86 ± 4.23	23.00 ± 0.09
Week 12	-	-	21.24 ± 3.19
Week 13	34.01 ± 2.49	31.74 ± 5.13	-
Week 39	20.33 ± 0.42	22.02 ± 2.83	-
Week 65	15.97 ± 2.33	15.36 ± 3.03	-
Week 91	10.57 ± 1.08	12.39 ± 3.08	-
Week 104	11.78 ± 0.97	11.74 ± 1.64	-

Table from data on pages 30-32 of MRID 42007502.

It is concluded that overall there is sufficient metabolism data (including excretion, distribution, retention half-life and amounts retained within different organs). This metabolism study in the rat, taken with previously submitted metabolism studies (in MRIDs 00080235 and 42007502) is classified as acceptable. The combination of these studies is adequate to satisfy the 85-1 data (metabolism study) guideline requirement.

Groups of male Sprague-Dawley rats received a single dose (0.2 mg/kg bw) of brodifacoum, bromadiolone, or flocoumafen by gavage. A control group consisting of 9 male rats which received nothing was also included in the study. The results showed that the levels of brodifacoum in the liver declined very slowly during the duration of the study as indicated by the difference between day 1 (1.107 µg/g) and day 200 (0.539 µg/g). During the first 28 days after dosing, the decline of the liver concentrations of bromadiolone and flocoumafen was faster than that of brodifacoum as indicated by the $t_{1/2}$'s of these 3 chemicals at the first 28 days (brodifacoum: $t_{1/2}$, 63 days; bromadiolone: $t_{1/2}$, 17 days; flocoumafen: $t_{1/2}$, 6 days). The decline of the liver concentrations of these 3 test chemicals occurred in a "bi-exponential manner". The second $t_{1/2}$'s were estimated to be 282, 318, and 159 days for brodifacoum, bromadiolone, and flocoumafen, respectively. In general, oral administration of any of these 3 chemicals would result in substantial retention of the chemical in the liver for a very long time. The initial report of this study contained deficiencies which were rectified in subsequent supplemental data submission. This study satisfies the data requirement for a modified metabolism study on bromadiolone (Guideline No. 85-1; MRID No. 42596801).

(2) Bromadiolone Metabolism

Groups of male Sprague-Dawley rats received a single dose (0.2 mg/kg bw) of brodifacoum, bromadiolone, or flocoumafen by gavage. A control group consisting of 9 male rats which received nothing was also included in the study. The results showed that the levels of brodifacoum in the liver declined very slowly during the duration of the study as indicated by the difference between day 1 (1.107 µg/g) and day 200 (0.539 µg/g). During the first 28 days

after dosing, the decline of the liver concentrations of bromadiolone and flocoumafen was faster than that of brodifacoum as indicated by the $t_{1/2}$'s of these 3 chemicals at the first 28 days (brodifacoum: $t_{1/2}$, 63 days; bromadiolone: $t_{1/2}$, 17 days; flocoumafen: $t_{1/2}$, 6 days). The decline of the liver concentrations of these 3 test chemicals occurred in a "bi-exponential manner". The second $t_{1/2}$'s were estimated to be 282, 318, and 159 days for brodifacoum, bromadiolone, and flocoumafen, respectively. In general, oral administration of any of these 3 chemicals would result in substantial retention of the chemical in the liver for a very long time. The initial report of this study contained deficiencies which were rectified in subsequent supplemental data submission. This study satisfies the data requirement for a modified metabolism study on bromadiolone (Guideline No. 85-1; MRID No. 42596801).

(3) Bromethalin Metabolism

A metabolism study was conducted in Fischer 344 rats following oral administration of ^{14}C-bromethalin at 1 mg/kg. Blood samples were taken from the orbital sinus at 0.25, 0.5, 1, 2, 4, and 24 hours, and at 2, 3, 4, 6, 8, 11, 14, 17, and 21 days after dosing. Based on radiolabeled material, the plasma half-life was 134 hours (5.6 days). The half-life of the distributive phase suggested distribution in total body water. The T ½ of bromethalin is 5.6 days. The major metabolite formed in the rat is desmethyl bromethalin. The study (MRID 0004724) was classified as acceptable.

(4) Diphacinone and its sodium salt Metabolism

In a metabolism study (MRID 92049009), the disposition of 14-C diphacinone was studied in Sprague-Dawley rats, Swiss albino mice, and Diphacinone-tolerant Norway rats. Sprague-Dawley rats received single oral doses of 0.18, 0.4 mg/kg (group A, 2 rats/group), 0.5, or 1.0 mg/kg (group B, 1 rat/group) labeled diphacinone and urine and feces collected up to 3 days post-dose (group A) or 8 days post-dose (group B). Swiss mice (1 mouse/group) received 0.6 mg/kg labeled diphacinone by oral intubation or sandwich method and urine and feces collected up to 4 days post-dose. Norway rats (1 rat) received 4 consecutive doses of 3.5 mg/kg labeled diphacinone and 1 dose of 6.1 mg/kg labeled diphacinone. Blood samples were obtained at 4, 6, 7, and 8 hours after the last dose and at 26 hours (time of sacrifice). A group of 10 diphacinone-tolerant Norwegian rats received 1.5 mg/kg labeled diphacinone in DMSO daily for 2-3 days to obtain enough excreta for metabolite identification. Because 4 different batches of labeled diphacinone were prepared for this study (label in different positions for each batch), eight Sprague-Dawley rats (2 females/group) received approximately 42 μg of labeled diphacinone from each labeled batch as a single oral dose, and TLC autoradiograms from these 4 labels were compared.

Absorption appeared fairly rapid but was only estimated in one rat, as judged by the blood levels measured over time. Distribution data showed that the liver, muscle, blood, fat, and lung were the tissues demonstrating the greatest retention of the test chemical in the order liver (14-25% of the dose), muscle (0.18-4.4% of the dose), fat (0.55-1.16% of the dose), and lung (0.04-0.5% of the dose). Rats appeared to show greater retention than mice of test chemical, but data were inconclusive based on limited numbers of animals and poor experimental design.

Half-life, as estimated in the single Norway rat, was stated as 17 hours, but is likely an underestimation. Elimination of diphacinone derived radioactivity was primarily in feces, with between 47-77% of the dose in feces of rats, and 69-73% of the dose in mice. At least five metabolites of diphacinone were identified in urine, feces, and/or liver. These metabolites represent hydroxylated products of diphacinone occurring on the phenyl and indandionyl rings.

This study is classified as unacceptable and does not satisfy the guideline requirement 85-1 for a metabolism study in rats. The unacceptable classification is based on the following deficiencies observed in this study:

1) Inadequate number of animals per dose group.

2) Four different radiolabeled parent compounds were administered in this study. Distribution data show that blood, liver, muscle and fat contained the highest amount of radioactivity, but the percentages found might depend on the label position. The excretion of only two different radiolabeled compounds was followed to any degree.

3) Inadequate experimental design for analysis of half-life.

4) Inadequate data on recovery of radioactivity from dosed animals.

5) No stated rationale for doses used.

The Agency requires metabolism data more adequately defining the half-life of diphacinone in the rat, as well as retention data for the liver.

(5) Chlorophacinone Metabolism

Agency records indicate only one metabolism study (MRID 00155540) on chlorophacinone has been received. Several experiments were conducted, including blood kinetics (2 experiments with a determination of radioactivity in organs 4 and 48 hours following dosage; urinary, fecal and biliary excretion).

In the first blood kinetics assay, four rats each received orally 1 mg of ^{14}C-labeled chlorophacinone. The following mean blood concentrations were measured as outlined in Table 25 below:

Table 25 - Mean blood concentration of chlorophacinone (in µg equivalents) following oral administration of 1 mg chlorophacinone

	30 min.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	24 hr.	48 hr.
Mean Blood Conc.	1.4	2.4	4.1	6.4	6.4	5.9	1.8	0.3

Chromatography and autoradiography demonstrated that the chlorophacinone remained unchanged in plasma, with a blood half-life of about 10 hours.

Organs of the rats used in the first blood kinetics study were assayed for radioactivity. The following results were obtained as outlined in Table 26 below:

Table 26 - Mean concentration of chlorophacinone (µg/g of organ)

Organ	4 hours	48 hours
Liver	31.1	2.9
Kidney	6.6	1.2
Lung	4.5	0.4
Heart	3.1	0.2
Muscle (thigh)	2.0	0.1
Fat	1.2	0.7
Carcass	5.2	0.3

In a second blood kinetics study, two rats each received 1.43 mg of ^{14}C-labeled chlorophacinone/day for 3 days. Blood samples were taken at various times following the third dose, with the following blood concentration measurements as outlined in Table 27 below.

Table 27 - Mean blood concentration of chlorophacinone (in µg equivalents) following three daily oral administrations of 1.43 mg chlorophacinone

	30 min.	1 hr.	2 hr.	4 hr.	6 hr.	8 hr.
Mean Blood Conc.	7.1	8.9	10.2	11.5	12.2	14.2

In an elimination assay, two rats were used. One received 1.43 mg of ^{14}C-labeled chlorophacinone and the second received 1.28 mg ^{14}C-labeled chlorophacinone. Daily assays were made of urine, feces and CO_2 for four days. The rats were sacrificed and radioactivity was measured in blood, organs and carcass. Urine and feces were extracted and measured by TLC and autoradiography.

Urine and CO_2 radioactivity were less than 1% of the total dose. Most of the radioactivity was excreted in the feces (94.7% in one rat and 108.6% in the other over the 4-day period). Excretion reached 90% in the first two days.

In a biliary excretion assay, two rats were used. Each received 1.4 mg of chlorophacinone intraduodenally. Bile was collected for 8 hours and total radioactivity was measured. TLC and autoradiography were performed on the bile directly before and after hydrolysis with glucuronidase.

Two hours after administration of chlorophacinone in the duodenum, biliary elimination was constant. At the end of 8 hours, an average of 26% of the administered radioactivity was eliminated in the bile.

The information provided in MRID 00155540 adequately addresses the guideline requirements 85-1 for a metabolism study for a highly toxic anticoagulant with no chronic

exposure. Although it is reported that there is over 90% excretion in the two days following dosage, the findings in the subchronic study (MRID 92018013) indicate there is a potential for bioaccumulation (or cumulative toxicity). In the subchronic study, there were mortalities at 40 µg/kg/day in 10/10 males (deaths occurred days 29-82) and 4/10 females (deaths on days 69-111), and there were also mortalities at 20 µg/kg/day in 4/10 males (deaths on days 105-111).

h. Other Toxicological Considerations

(1) Brodifacoum - Other Toxicological Considerations

In an antidotal study (MRID 42007501), four male beagle dogs each received a single oral dose of 5 mg/kg brodifacoum (96.8%). Prothrombin times for each of the dogs were then monitored over a period of five weeks. "Doses of 2 mg/kg vitamin K_1 were administered to dogs by the intramuscular route whenever their prothrombin times were elevated to levels consistent with a life-threatening effect on coagulation." Individual dogs required 12-15 vitamin K_1 treatments in the period from days 2 to 29 post-dosing. All four dogs survived to the end of this study (5 weeks after the test material was administered). However, based on elevations in prothrombin time, vitamin K_1 had to be administered to one dog on day 29. This dog had also been treated with vitamin K_1 on days 23 and 24 as well as on previous occasions, and the last prothrombin time measurement for this dog was on day 34. The possibility exists that this dog would have required additional vitamin K_1 treatments after day 34.

(2) Bromadiolone - Other Toxicological Considerations

In an antidotal treatment study, groups of male Crl:CD^R rats (10/dose) were exposed to bromadiolone baited pellets (0.005% a.i.) for 24, 48, or 78 hours. The estimated mean total bromadiolone doses were 5.69, 9.76, and 15.63 mg/kg for 24-, 48-, and 72-hour groups, respectively. At the end of the exposure period, the first 5 surviving rats of each group were given vitamin K_1 at 5 mg/kg. Initially, a loading dose was given subcutaneously, and subsequently, vitamin K_1 was administered daily by gavage for 13 days. The survivors were sacrificed at 8 to 10 days after discontinuing the vitamin K_1 treatment.

The animals which did not receive vitamin K_1 died in each exposure group. The deaths frequently occurred within 3 to 4 days of the study. The clinical and gross pathology findings were hemorrhage-related toxicity in all test-article treated animals. The death rates in vitamin K_1 treated animals were 1/5, 2/5, and 5/5 in the 24-, 48-, and 72-hour exposure groups, respectively. With vitamin K_1 treatment, the clinical findings (hemorrhagic-related toxicity) were resolved by the 5^{th} day of the antidote treatment, and the decrease in body weight observed during the bromadiolone treatment was also restored in the surviving animals. At the 2^{nd} week of the study, the prothrombin times of the vitamin K_1 treated animals were essentially comparable to those of the controls. However, for the 48-hour exposure group, the prothrombin time was slightly decreased relative to that of the control.

The results demonstrate that vitamin K_1 treatment, as employed in this study, can restore the clotting process of an animal which is exposed to bromadiolone below an estimated total dose of 15.63 mg/kg body weight during a 72 hour period. However, the antidotal treatment may not completely prevent death (i.e. all the rats died in 72-hour exposure groups with vitamin K^1

treatment) when rats are exposed to bromadiolone even at the lowest exposure dose (5.69 mg/kg) in this study. This study satisfies the data requirements for an antidotal study (Guideline 86-1; MRID No. 42093301).

(3) Bromethalin - Other Toxicological Considerations

The following information is in the Agency's files and are supportive of the endpoint of toxicological concern identified in the above studies.

Ph.D. Dissertation entitled "Bromethalin-Based Rodenticides: Mode of Action, Toxicity, Clinical Effects, and Treatment Efficacy in Rats, Dogs, and Cats", by D. Dorman, University of Illinois, Dept. of Veterinary Biosciences (MRID 42759602).

This dissertation is a summary of information found in the literature. According to the summary page of the dissertation, "The purpose of these studies was to define the toxicity of bromethalin-based rodenticides, develop treatments, and determine new modes of action of bromethalin….. Sublethal doses of bromethalin to dogs and cats resulted in delayed CNS depression, hind-limb ataxia, paresis, and paralysis. Higher doses given to dogs resulted in rapid severe muscle tremors and generalized seizures. Bromethalin toxicosis was also associated with increased cerebrospinal fluid pressure and cerebral edema. Bromethalin toxicosis produced acute and chronic EEG changes. Predominant abnormal EEG changes included spike and spike-and-wave EEG patterns; high voltage slow wave activity; photoconvulsive or photoparoxysmal irritative responses, and marked voltage depression. Histologic lesions included diffuse white matter spongiosis, mild microgliosis, and optic nerve vacuolization. Ultramicroscopic examination of brainstem revealed occasional swollen axons, intramyelenic vacuolization, and myelin splitting at the intraperiod line."

The Toxicity and Mechanism of Action of Bromethalin (MRID 42795603).

This publication is a journal article with only summary data. The study authors state " Doses in excess of the LD_{50} (2 mg/kg in rats) will cause death within 8-12 hours and it is preceded by one to three episodes of clonic convulsions with death usually due to respiratory arrest. Multiple low doses or sublethal intoxication yield hind leg weakness and loss of tactile sensation in rodents. Histopathology of the brain and spinal cord of these animals revealed a spongy degeneration of the white matter which was shown upon ultramicroscopic examination to be intramyelenic edema. …Mechanistic studies showed that bromethalin is rapidly converted to the desmethyl analog which is an extremely potent uncoupler of oxidative phosphorylation. It was theorized that if this occurs in the central nervous system, a fluid imbalance may ensue due to insufficient adenosine triphosphate (ATP). Fluid buildup in the cranium was determined by measuring cerebrospinal fluid pressure (CSFP), brain and spinal cord moisture, and cation concentrations."

Toxicity and Efficacy of Bromethalin (MRID 42795604).

This report is a published journal article with no raw data. The study authors state "Acute oral LD_{50} values range between 1 and 13 mg/kg for several mammalian and avian species.

Results of experiments designed to determine the physiological and biochemical mechanism of action suggest that treatment with bromethalin results in the uncoupling of oxidative phosphorylation in central nervous system mitochondria."

(4) Chlorophacinone - Other Toxicological Considerations

In an antidotal study (MRID 41981101) groups of 10 male rats were offered pelleted end-use product (containing 0.005% chlorophacinone) as their sole dietary source of food for 24, 48 or 72 hours. Reported mean dose levels were 5.28, 4.73 and 5.03 mg chlorophacinone/day. About 1-2 hours after the end of their respective exposure periods, five males in each group received a subcutaneous injection of 5 mg/kg vitamin K_1, followed by vitamin K_1 by daily oral gavage (5 mg/kg/day) for the next 13 days. Animals were sacrificed 8-10 days after the last oral dose of vitamin K_1. Five animals in each dose group did not receive vitamin K_1 treatment.

All rats that ate the chlorophacinone-containing pellets and did not receive vitamin K_1 died. All rats treated with vitamin K_1 after 24-hour exposure to the chlorophacinone diet survived, as did 3/5 rats fed the chlorophacinone-containing diet for 48 hours. All of the vitamin K_1-treated rats which had been fed the chlorophacinone-containing diet for 72 hours died.

While vitamin K_1 has been shown to be a somewhat effective treatment following chlorophacinone ingestion, there were some mortalities among the rats which were given vitamin K_1 at 48 hours. This suggests a potential hazard if incidents occur involving pets or small children in which it is not known or realized that ingestion has occurred. It is noted that this antidotal study does not include prothrombin times. Such information, while not necessary for purposes of reregistration, could be useful to the Agency in defining hazards associated with exposure to chlorophacinone.

(5) Diphacinone and its sodium salt - Other Toxicological Considerations

A collection of published articles (in MRIDs 42791201, 42791202, 42791203) from the literature has been submitted to support the use of Vitamin K_1 as an antidote in treating diphacinone poisoning.

In one report (Mount, M. E. and B. F. Feldman, 1983. The Mechanism of Diphacinone Rodenticide Toxicosis in the Dog Clarified and Its Therapeutic Implications. Am. J. of Vet. Res. 44(11): 2009-2017; in MRID 42791201) the clinical effectiveness of Vitamin K_1 therapy in reversing anticoagulant effects of two rodenticides in male dogs was investigated. Warfarin and diphacinone were administered in the diet twice daily for 3 days. Warfarin was fed to one dog at a total dose of 5 mg (a.i.)/kg and diphacinone was fed to three dogs at 2.5 mg (a.i.)/kg. These doses would generally be lethal for repeated exposure. Evidence of coagulopathy was observed by day 3, and Vitamin K_1 therapy was initiated for all dogs on day 6 at divided doses 3 times/day over a 5-day interval. One warfarin and one diphacinone-dosed dog received 2.5 mg K_1/kg/day, while the other two diphacinone-dosed dogs received 5 mg K_1/kg/day. All animals survived to the termination of the study.

A single regime of vitamin K_1 (2.5 mg/kg/day for 5 days) was effective in reversing hypoprothrombinemia of the warfarin-treated dog. However, this regimen was ineffective for diphacinone-treated dogs, which required either 5 mg vitamin K_1/kg/day administered in repeated doses 3 times/day on days 6-10 and 16-20, or 2.5 mg vitamin K_1 in repeated doses on days 6-10, 16-20 and 26-30. In addition, one diphacinone-dosed dog received fresh plasma with the first vitamin K_1 injection.

The three diphacinone-exposed dogs had prolonged bleeding at venepuncture sites the last day of treatment. All dogs became clinically ill within the subsequent 3 days. Bleeding was observed in the diphacinone-exposed dogs as long as 2 weeks following exposure. An important finding was that the vitamin K-enzyme complex was inhibited in diphacinone-exposed dogs for approximately 30 days as indicated by routine coagulation screen tests and coagulation factor inhibition. No hepatic dysfunction was observed. There was a statistically significant reduction ($p < 0.001$) in pancreatic exocrine function although the resulting values were within the laboratory's reference range.

According to the published paper in MRID 42791201:

> "The liver synthesizes the vitamin-K dependent coagulation proteins, factors II, VII, IX, and X, to inactive precursor forms dependent upon vitamin K for activation by a postribosomal protein modification. The inactive precursor proteins contain several glutamic acid residues which serve as the site for vitamin K function. These amino acids are carboxylated to form gamma-carboxyglutamic acid (G1a) residues which are responsible for activation of the coagulation protein. Calcium binding is dependent upon this cluster of carboxylic groups; without calcium binding the factor is nonfunctional. Hence, vitamin K serves as an essential cofactor for the enzyme that carboxylates protein-bound glutamic acid residues to G1a.
>
> The molecular role of vitamin K in the carboxylation event is unclear. The carboxylase enzyme has been studied and its activity measured. A reductase, epoxidase (carboxylase-epoxidase enzyme), and epoxide reductase enzymes are also closely associated with the metabolic role of vitamin K… This…collectively represents the vitamin K-enzyme complex. This term is used since the complete biochemical mechanisms of vitamin K metabolism are not completely understood. The site of the biochemical lesion caused by anticoagulant rodenticides is the epoxide reductase enzyme… The carboxylase-epoxidase enzyme interaction…is not understood but results in G1a formation and conversion of vitamin K to the inactive epoxide. The epoxide can then be reconverted to the vitamin K quinone through the epoxide reductase enzyme… Without this enzyme vitamin K cannot be recycled. This results in rapid depletion of body stores of vitamin K…"

The material in MRID 427912201 satisfies the guideline data requirement (§86-1) for an antidotal study.

Diphacinone (as "Dipaxin") has been investigated and used as a therapeutic anticoagulant in humans (Correll, J.; Coleman, L.; Long, S.; Willy, R., 1952). According to this report, a single oral dose of 16 mg given to a healthy man weighing over 200 lbs resulted in no significant change in prothrombin determinations over a period of 48 hours. A second man of similar weight was given 32 mg, and changes in the prothrombin time were evident within a period of 4 hours. The maximum effect was seen at about 16 hours, with decrease in prothrombin time at 30 hours, and recovery to normal at 70 hours. A third patient, a 95-lb 22-year-old man received 32 mg as

a single dose; definite increase of prothrombin time was measurable within 23 hours, and a "therapeutic" effect (prothrombin time > 15 seconds) was evident within 48 hours. Following this single dose, 12 days were required for the prothrombin time to return to normal range. The dosage was then repeated, and prothrombin time was prolonged to 31 seconds (normal is about 12 seconds) within 36 hours. The following day, the patient received 50 mg of vitamin K intravenously. One hour later the prothrombin time was 26 seconds; 2 hours later it was 19 seconds, and approximately normal prothrombin values had been restored at 7 hours.

Dipaxin was given to a 30-year-old woman with post-operative venous thrombosis. Definite prolongation of the prothrombin time (to approximately 22 seconds) developed 24 hours after a single 32-mg dose. Repetition of this dose on the third day brought prolongation of the prothrombin time into the optimal therapeutic range (24-36 seconds), where it was maintained by 5 mg of Dipaxin daily. On the day of the last dose (day 13) the prothrombin time was 26 seconds, returning to normal range 3-4 days later.

Additional information on clinical investigations of Dipaxin in humans are given in Katz et al. 1954 (in which it is stated that "The prothrombopenic action of Dipaxin is readily counteracted with vitamin K_1 administered either orally or intravenously) and Field et al. 1952 (in which it is stated that, in man: "This agent induces an effective hypoprothrombinemia in single doses of as little as 4 mg… After single doses of 20 mg a marked hypoprothrombinemia was usually evident in 48 hours which persisted from 6 to 10 days… The recommended starting dose is about 20 mg… The maintenance of adequate clinical hypoprothrombinemia was obtained with daily doses of 2 to 4 mg. Hypoprothrombinemia was readily overcome with vit. K_2, the natural vitamin being more effective than the synthetic…".

2. Exposure Assessment

a. Dietary Exposure

These chemicals are non-food use pesticides. Therefore, it is unlikely that there will be any exposure to food sources or to residues in ground or surface water contamination.

b. Occupational and Residential Exposure

The following assumptions were made:

- All formulations are 0.005% a.i. (note: some end-use products formulations have a higher percent a.i., but using these would make a comparison of MOEs more difficult). In order to calculate MOEs for a higher percent a.i. the calculations would be adjusted accordingly;
- A child weighs 10 kilograms; and
- Poison specialists estimate that a child would consume approximately 5 grams in one bite.

These assumptions were extracted from the various rodenticides in this RED.