Appendix D

HED Toxicity Profile for Bromadiolone

(from the 1998 Rodenticide Cluster RED)

(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).

(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.

(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).

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.

(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).