Appendix E

HED Toxicity Profile for Chlorophacinone

(from the 1998 Rodenticide Cluster RED)

(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.

(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).

(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.

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.

(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).