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Attachment D

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003478

DATA EVALUATION REPORT

Study Type: One-Year Oral Toxicity Dog (83-1)

TOX Chem. No. 003B
MRID No.:41565 1-18
Project No. 0-1999

Test Material: N-ethoxymethyl-N-(2-methyl-6-ethylphenyl) chloroacetamide

Classification: Preemergence Herbicide

Common Name: Acetochlor, SC-5676

Study No.: LSR Report No. 88/SUC018/0136

Date of Study: December 2, 1988

Sponsor: Imperial Chemical Industries, Inc.

Testing Facility: Life Science Research, Ltd.

Title of Report: SC-5676: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 Weeks.

Author: Alan Broadmeadow

Quality Assurance: D.L.M. Weller

Conclusion: Classification of Data: Guideline

This study satisfies the guideline data requirement (83-1) for a chronic nonrodent oral toxicity study.

This study meets the criteria of 40 CFR 158.34 for neurotoxicity (6) at the 50 mg/kg/day level (HDT).

NOEL = 2 mg/kg/day

LEL = 10 mg/kg/day with salivation, significant increase in alanine aminotransferase and ornithine carbamyl transferase activity accompanied by a significant increase in triglyceride and decreased blood glucose values.

Histopathological changes at this level were in the kidneys (interstitial nephritis and chronic vasculitis), testes (tubular degeneration), epididymides (hypospermia) and liver (reduced glycogen).

A. Materials:

1. Test Compound - Technical SC-5676 of batch one and three with a purity of 91 percent was used in the study. The test material, a dark brown viscous liquid, was stored in an amber glass container for preparation of the gelatin capsules. Analysis of SC-5676 at the conclusion of the study indicated a concentration of 90.5 percent.

2. Test Animals - Twenty male (8.6 to 11.2 kg) and 20 female (8.0 to 10.9 kg) 20 week old pure-bred beagle dogs were used in this study.

B. Study Design:

1. Allocation of Animals:

Dose Level (mg/kg/day)	Male	Female
Control	5	5
2.0	5	5
10.0	5	5
50.0	5	5

The selection of dose levels for this study was based on a LEL of 60 mg/kg/day demonstrated in a 90-day oral toxicity study in dogs (MRID No. 415651-16).

All animals were housed individually with temperature and humidity controlled to provide a uniform environment. A 12-hour light/dark cycle was provided. Each dog received 400 g of a dry pelleted diet each morning before treatment. The uneaten food was withdrawn and weighed the following morning. Water was available ad libitum. All dogs were vaccinated against hepatitis, leptospirosis, distemper and canine parvovirus at 6, 9 and 12 weeks of age and against Bordatella bronchiseptica at 12 weeks of age.

2. Treatment - The liquid test material was weighed into size 00 gelatin capsules based on the most recent recorded bodyweight. The capsules were administered orally once each day after feeding, seven days a week for 52 weeks. Control animals received empty size 00 capsules.

3. Statistical - The significance of intergroup differences in body weight change, hematology, clinical chemistry, and urinalysis was assessed by Student's t-test using a pooled error variance. The significance of intergroup differences in organ weights was assessed by Dunnett's test.

C. Methods and Results:

1. Observations - Individual daily observations were recorded before and after each dose. In addition, the animals were observed periodically during the day for signs of toxicity.

a. Gross observations include excessive salivation observed within 30 minutes to five hours after dosing and abnormal shaking of the head associated with salivation in the high-dose males and females. The incidence of excessive salivation among males of the mid- and high-dose levels was less frequent and less severe than observed in the high-dose female dogs during the study. Males and females of the high dose level appeared emaciated during the latter half of the study.

b. Neurological examination, as a consequence of the signs of toxicity observed, was performed after 47 weeks with the following parameters evaluated:

Cranial nerve reflex
- Pupillary light and consensual light
- Palpebral-blink and corneal
- Gag
- General examination of the head

Segmental reflex
- Flexor (withdrawal) and crossed extensor
- Patellar
- Extensor tone

Postural reactions
- Placing-visual and tactile
- Extensor postural thrust
- Righting
- Tonic neck reactions
- Hopping reflex

General observations
- Behavior changes
- Abnormalites of gait and stance
- Tremor or other dyskinesis

Neurological changes were observed at the 50 mg/kg level during the last six weeks of the study. These changes were severe in 2/5 male and 3/5 female dogs. Neurological changes were less severe in 2/5 males and 1/5 females and not apparent in 1/5 males and 1/5 females at the 50 mg/kg dose level. These neurological changes comprised of "swaying or shaking of the body and head, head oscillation, stiffness and rigidity of the hindlimbs resulting in incoordination, ataxia, tremor and high-stepping gait, depressed righting, hopping and flexor reflexes and exaggerated tonic neck reflex". The two males exhibiting these neurological changes were killed during week 46 and the four females during week 39*, 46, 48, and 51 of the study. These neurological changes were not appearent at the mid and low levels.

c. Mortality - Six animals exhibiting neurological changes at the 50 mg/kg level were killed during the last six weeks of the study.

Dose Level (mg/kg)	Males	Females
Control	0/5	0/5
2.0	0/5	0/5
10.0	0/5	0/5
50.0	2/5	4/5

*A neurological examination was not performed for this female dog, however, this animal was ataxic prior to death during week 39.

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d. Food Consumption - Each dog received 400 g of a dry pelleted diet each morning before treatment and the uneaten food was withdrawn and weighed the following morning.

A 23 to 28 percent decrease in food consumption was observed for females dosed the 50 mg/kg level during the last 39 weeks of the study. At the 10 mg/kg level a 13 percent decrease in food intake was observed for females during the last 13 weeks of the study. Food consumption of the low dosed females and the three test levels for males were comparable to their respective control values for the 52-week period.

e. Water intake was monitored following an increase in urinary volume after 24 weeks of treatment. "Water consumption was recorded over a three day period using polyethylene bottles fitted with Lixit valves" during weeks 30, 39, and 52.

An increase in water intake was observed during weeks 30-52 at the 50 mg/kg level in males and females by 57 and 23 percent, respectively, as compared to the control values.

f. Body Weight - All animals were weighed weekly before feeding and then prior to necropsy regardless of feeding cycle.

A significant ($p < 0.001$) decrement in body weight change was reported for the high dose females during weeks 26 and 39 by 90 and 81 percent, respectively. A decrease in food intake of 23 to 28 percent was observed during this period.

A significant ($p < 0.05$) decrement in body weight change was reported by the 39th week for the high dose males by 65 percent.

Mean body weight (Kg) values and percent change (%) as compared to the control values are presented in the following table.

	Male (mg/kg)				Female (mg/kg)			
Week	0	2.0	10.0	50.0	0	2.0	10.0	50.0
13	2.6	2.1(19)	2.1(19)	1.7(35)	2.3	2.3(0)	2.0(13)	1.5(35)
26	3.0	2.1(30)	2.4(20)	1.5(50)	3.1	3.0(3)	2.5(19)	0.3(90)**
39	3.1	2.1(32)	2.3(26)	1.1(65)*	3.7	3.3(11)	3.2(14)	0.7(81)**
52	3.2	2.4(25)	2.7(16)	2.2(31)	4.1	3.6(12)	3.4(17)	0.1(98)

* $p < 0.05$
** $p < 0.001$

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g. Ophthalmoscopy examinations were performed initially, at 24 and 50 weeks of the study.

No treatment-related findings were observed between the treatment and control groups.

2. Clinical Findings - Blood was collected for hematology and clinical chemistry prior to administration of the oral dose from animals fasted overnight. Blood samples were collected one week before the initial dose, then after the 12th, 24th, and 50th week of the study. In the study report, terminal hematology and clinical chemistry values from the decedent animals were reported separately from the animals surviving 50 weeks of treatment. The clinical findings of the decedent animals were not included in this Data Evaluation Report.

a. Hematology parameters examined: The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Erythrocyte count
* Hemoglobin
  Erythrocyte sedimentation rate
* Hematocrit
  Reticulocyte count
  Prothrombin time
  Activated partial thromboplastin time
* Leucocyte count
* Leucocyte differential count
* Platelet count
  Mean corpuscular hemoglobin
  Mean corpuscular volume
  Mean corpuscular hemoglobin concentration

These animals were not Factor VII deficient as reported in the subchronic dog study (MRID 415651-16).

No dose related hematological findings were reported for those animals that survived 50 weeks of compound administration.

b. Clinical chemistry parameters examined: The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Alkaline phosphatase (AP)
* Alanine aminotransferase (ALT)
* Aspartate aminotransferase (AST)
  Cholinesterase-plasma and RBC
  Sorbitol dehydrogenase (SDH)
  Ornithine carbamyl transferase (OCT)
  Gamma-glutamyl transpeptidase (GGT)
* Urea
* Creatinine
* Glucose
* Albumin
* Creatine phosphokinase (CPK)
* Total bilirubin
  Total triglyceride
* Total cholesterol
* Total protein
  Electrophoretic protein
* Sodium
* Chloride
* Potassium
* Inorganic phosphorus
* Calcium

Parameter recommended but not reported: lactic dehydrogenase

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Alanine aminotransferase (ALT) activity was significantly elevated in excess of 100 percent at the 50 mg/kg level, as compared to the control values, in males ($p < 0.05$) during weeks 12, 24, and 50 and in females ($p < 0.01$) during weeks 12 and 24 of the study. Increased ALT activity was observed at the 10 mg/kg level in males by 32, 19, and 70 percent during weeks 12, 24 and 50, respectively. Female ALT activity was significantly ($p < 0.01$) elevated at the 10 mg/kg level in excess of 100 percent at the termination of the study.

Gamma-glutamyl transpeptidase (GGT) activity was significantly ($p < 0.05$) elevated in males at the 50 mg/kg level by 100, 25, and 75 percent during weeks 12, 24, and 50, respectively.

Ornithine carbamyl transferase (OCT) activity was significantly elevated in excess of 100 percent at the 50 mg/kg level in males ($p < 0.01$) during weeks 12, 24, and 50 of the study and in females ($p < 0.05$) at the 10 mg/kg level by week 50.

Blood urea values were elevated at the 50 mg/kg level significantly in males ($p < 0.01$) by 58 and 80 percent during weeks 24 and 50, respectively, and in females ($p < 0.05$) by 93 percent at the termination of the study.

Blood creatinine values were elevated at the 50 mg/kg level significantly in males ($p < 0.001$) by 50 percent at the termination of the study and in females ($p < 0.05$) by 33 and 83 percent during weeks 24 and 50, respectively.

Blood glucose values were decreased at the 50 mg/kg level significantly in males ($p < 0.05$) by 10 and 14 percent during weeks 12 and 24, respectively, and in females ($p < 0.05$) by 11 to 14 percent during weeks 12, 24, and 50 of the study. Female blood glucose values were significantly ($p < 0.05$) depressed at the 10 mg/kg level by 7 and 9 percent during weeks 24 and 50, respectively. In addition, female blood glucose values were significantly ($p < 0.01$) decreased at the low level during week 50 by 8 percent.

Triglyceride values at the 50 mg/kg level were significantly ($p < 0.01$) elevated in males by 46, 59, and 67 percent, during weeks 12, 24, and 50, respectively. In females the triglyceride values were significantly ($p < 0.05$) elevated by the 12 week interval (only) at the 10 and 50 mg/kg levels by 34 and 38 percent, respectively.

Cholesterol values at the 50 mg/kg level were significantly ($p < 0.05$) elevated in males by 26, 40, and 36 percent during weeks 12, 24, and 50, respectively.

Plasma cholinesterase values were significantly ($p < 0.05$) elevated in females at the 50 mg/kg level for acetyl activity during week 24 by 34 % and for butytyl activity during weeks 12 and 24 by 17 and 32%, respectively.

A dose related trend was observed for an increased acetyl and butyryl activity in females at the 10 mg/kg level. Male plasma cholinesterase values at the 50 mg/kg level were elevated during weeks 24 and 50 for for acetyl activity by 19 to 23% and for butyryl activity by 22%.

A summary of the statistically significant blood chemistry changes reported for the mid- and high-dose levels are presented in the following table.

Sex	Male						Female					
Dose (mg/kg/day)	10			50			10			50		
Interval (weeks)	12	24	50	11	24	50	12	24	50	12	24	50
Parameter:												
Alanine aminotransferase				↑	↑	↑			↑	↑	↑	
Gamma-glutamyl transferase				↑	↑	↑						
Ornithine carbamyl transferase				↑	↑	↑			↑			
Urea					↑	↑						↑
Creatinine						↑					↑	↑
Glucose				↓	↓			↓	↓	↓	↓	↓
Triglyceride				↑	↑	↑	↑			↑		
Cholesterol				↑	↑	↑						
Plasma cholinesterase acetyl											↑	
butyryl										↑	↑	

c. Urinalysis - Urine was collected prior to the initial treatment then during weeks 11, 23, and 49 from animals housed individually in metabolism cages overnight without food or water. The following were examined: The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Appearance	* Protein	* Bilirubin
* Volume	Reducing substances	Nitrites
pH	* Glucose	* Blood
* Specific gravity	* Ketones	* Microscopic sediment

An increase in urinary volume was reported at the 50 mg/kg level for females and significantly ($p < 0.05$) for males during weeks 23 and 49. This increased urinary volume was accompanied by a decrease in specific gravity and appearence "considered to be paler than normal".

d. Fecal examination for occult blood - Feces were collected over a three day period initially then during weeks 12, 24, and 50.

Feces from male and female dogs dosed at 2, 10, and 50 mg/kg were negative for blood.

3. Terminal Observation - On completion of the experimental period, all animals were anesthetized with sodium pentobarbital and killed by exsanguination. The following tissues were collected for histopathological examination and the (X) checked organs were weighed. Organ weights for decedent animals were included for the calculation of group means and standard deviations. The checked (*) parameters were recommended by Subdivision F guidelines of November 1989.

*	X	Adrenals	*	X	Liver	*	Skeletal muscle
		Aorta (thoracic)	*	X	Lung	*	Skin
*	X	Brain	*		Lymph-cervical	*	Spinal Cord -
		Bronchi			mesenteric		Cervical, thoracic,
*		Caecum			peribronchial		and Lumbar
		Colon	*		Mammary Glands	*	X Spleen
*		Duodenum			Nasopharynx	*	Sternal bone and marrow
*		Epididymides	*	X	Ovaries	*	Stomach - Pylorus
*		Esophagus	*		Pancreas		and fundus
*		Eyes and optic nerve			Para-nasal sinus	*	X Testes
		Femur	*	X	Pituitary	*	Thymus
		Gallbladder		X	Prostate	*	X Thyroid and
*	X	Heart	*		Rectum		parathyroid
*		Ileum	*		Salivary Gland		Tongue
		Jejunum			Sciatic Nerve	*	Trachea
*	X	Kidneys					Turbinates
						*	Urinary Bladder
						*	X Uterus
							Vagina

a. Brain Cholinesterase - Brain tissues taken at necropsy from the mid-line region of the cortex and both hemispheres were examined by the Ellman method. No dose-relared changes were reported in brain acetyl cholinesterase activity at the dose levels tested.

b. Organ Weights - Terminal body weights were reduced at the 50mg/kg level as compared to the controls for males by 16 % and significantly ($p < 0.01$) for females by 37%. Statistically significant changes in relative organ weights were limited to animals at the 50 mg/kg level. Male and female relative kidney weights were comparable to their respective control values. However, a significant ($p < 0.01$) decrease in absolute kidney weight of 37% was reported for females at the 50 mg/kg level.

Female relative organ weights at the 50 mg/kg level were significantly ($p < 0.01$) increased for brain and heart by 55 and 27%, respectively. Male absolute heart weights were significantly ($p < 0.05$) reduced by 14 % at the high dose level.

At the 50 mg/kg level relative adrenal weights were increased for males by 28 % and significantly ($p < 0.01$) for females by 78 %.

Relative liver weights at the 50 mg/kg level were increased for females by 17 percent and significantly ($p < 0.01$) for males by 32 %. Female absolute liver weights were significantly ($p < 0.05$) reduced by 27% at the high dose level.

A significant increase in relative lung weights was reported at the 50 mg/kg level for males ($p < 0.05$) and females ($p < 0.01$) by 16 and 32 %, respectively. Female absolute lung weights were significantly reduced at the 10 ($p < 0.05$) and 50 ($p < 0.01$) mg/kg levels by 13 and 19%, respectively.

At the 50 mg/kg level a significant decrease in relative ($p < 0.05$) and absolute ($p < 0.01$) testes weight was reported by 34% and 48%, respectively. Relative thyroid weights of males at the 50 mg/kg level were significantly ($p < 0.05$) increased by 28%.

The percent increase and/or decrease in relative organ and absolute ( ) weights, for those respective organs that demonstrated a statistically significant change at the 50 mg/kg level, are presented in the following table.

	% Increase						% Decrease				
	Adrenal	Brain	Heart	Liver	Lung	Thyroid	Heart	Kidney	Liver	Lung	Testes
Male				32	16	28	(14)				34(48)
Female	78	55	27		32			(37)	(27)	(19)	

c. Macroscopic pathology findings were limited to the 50 mg/kg level. The animals killed prematurely were emaciated in appearence. The kidneys of males and females were abnormal in shape with multiple pale areas on the surface. Cervical lymph nodes of males (3/5) and females (3/5) were dark in appearance. The gallbladder was distended in males (1/5) and females (2/5). Adrenals were enlarged in 2/5 females. The liver of 2/5 males was pale in appearence.

d. Histopathological Examination - Treatment-related histopathological changes in the brain and kidneys of both sexes and in the testes of males were reported for the 50 mg/kg level. In the cerebellum, there were degeneration of the granular layer and depletion of the Purkinje cells. Kidney changes consisted of collecting duct hyperplasia, transitional cell hyperplasia, cortical atrophy with fibrosis and scarring accompanied by chronic vasculitis, interstitial nephritis, dilatation of Bowman's space and lipofusin pigment in the cortical tubules.

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Testicular findings consisted of the degeneration of seminiferous tubules, maturation arrest and the formation of spermatid giant cells within the tubules. Hypospermia in the epididymides was also reported at the high-dose level.

Histopathological changes at the 10 mg/kg level were limited to the kidneys, epididymides, and testes of males. Kidney changes consist of interstitial nephritis, and chronic vasculitis. Hypospermia of the epididymides and seminiferous tubule degeneration were reported at the mid-dose level.

A dose related decrease in glycogen content in the liver was reported for males of all three dose levels and females of the mid and high dose levels.

The following table (from this study, table 11, pages 102-114) summarizes the the incidence of histopathological findings in the test groups of a greater frequency than observed in the control animals. Two males and four females at the 50 mg/kg level were killed during the experimental period.

Sex	Male				Female			
Dose (mg/kg/day)	0	2	10	50	0	2	10	50
Brain, Vermis cerebellum				4/5				3/5
Degeneration of the granular layer				4/5				2/5
Depletion of Purkinje cells								
Demyelination and degeneration of granule cell axons				1/5				
Kidney			2/5	5/5	1/5			4/5
Interstitial nephritis				5/5				5/5
Collecting duct hyperplasia			3/5	4/5				5/5
Chronic vasculitis				4/5				5/5
Cortical fibrosis and/or scarred areas				4/5				4/5
Dilatation of Bowman's space				4/5				5/5
Cortical atrophy				4/5				3/5
Lipofuchsin pigment in cortical tubules		1/5		1/5				2/5
Dilatation of capilary blood vessels				1/5				2/5
Collecting duct cortical dilatation				5/5				5/5
Transitional cell hyperplasia								1/5
Papillary necrosis								1/5
Focal necrosis								
Liver		1/5	2/5	4/5	1/5		1/5	4/5
Reduced glycogen				1/5				1/5
Pigment in hepatocytes								
Epididymides		1/5	2/5	5/5				
Hypospermia								
Testes			4/5	5/5				
Tubular degeneration				5/5				
Maturation arrest		1/5		4/5				
Spermatial giant cells within tubules								

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Conclusion: The toxicity of SC-5676, when administered orally in capsules to dogs at 2, 10 or 50 mg/kg for 7 days per week for 52 weeks, was evident by treatment related changes in the brain, kidney and testes of animals dosed at the high level. Renal toxicity was manifest after 24 weeks of treatment at the 50 mg/kg level by increased water intake, urinary volume, blood urea and creatinine values. Alterations in kidney macroscopic and microscopic pathological findings were reported at the termination of the study. Neurological effects were appearent after 39 weeks of treatment at the 50 mg/kg level by changes in posture, gait, reflexes and ataxia. Two males and four females were killed between weeks 39 and 51 due to the severity of the neurological effects. These neurological changes were associated with the histopathological findings in the vermis cerebellum. Testicular effects consisted of decreased relative testes weight, atrophy and alterations in histopathological findings at the 50 mg/kg level. Increased clinical chemistry values, increased relative liver weight and reduced glycogen content characterized the liver effects. In addition, animals at the high dose level exhibited excessive salivation and weight loss (emaciation).

NOEL = 2 mg/kg/day

LEL = 10 mg/kg/day with salivation, significant increase in alanine aminotransferase and ornithine carbamyl transferase activity accompanied by a significant increase in triglyceride and decreased blood glucose values.

Histopathological changes at this level were in the kidney (interstitial nephritis and chronic vasculitis), testes (tubular degeneration), epididymides (hypospermia) and liver (reduced glycogen).

Classification of Data: Guideline

This study satisfies the guideline data requirement (83-1) for a chronic nonrodent oral toxicity study.

This study meets the criteria of 40 CFR 158.34 for neurotoxicity (6) at the 50 mg/kg level (HDT) with neurological behavioural changes, death of 2/5 males and 4/5 females between weeks 39 and 51 due to the severity of the neurological effects and neuropathology reported at this level.