3-22-82

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

001544

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: March 22, 1982

SUBJECT: CIPC; EPA Reg.#748-161, 748-163; Miscellaneous Data CASWELL#510A Acc.#246648-650

FROM: William Dykstra, Toxicologist
Toxicology Branch/HED (TS-769) WHD FDC 3/23/82

TO: Robert Taylor (25)
Registration Division (TS-767) Hfm RSP

Recommendations:

1. The acute delayed neurotoxicity study was negative for CIPC.

2. There are several unexplained questions regarding the mutagenicity studies.

   1) Ames Assay: Study I

      a. Strains TA1538 and TA100 were not tested.

      b. There were unexplained asterisks in Table 7 indicating values which appear to be positive (i.e., increased mutagenicity at higher levels).

   2) Ames Assay: Study II

      a. Strains TA1538 and TA1535 were not tested.

      b. The dosages used were not high enough.

   3) BALB/C 3T3 Transformation Assay

      a. There is no indication of positive controls.

      b. There is no indication of metabolic activation.

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3) The references cited regarding the potential of CIPC to be an inhibitor of cholinesterase activity are required to be submitted.

4) The registrant is required to submit the composition of the CIPC 40.4% formulation (Lot#237-1283). Additionally, a complete final report of the teratology study which includes the full results obtained by consultants to PPG as well as the unchanged Cannon Laboratory report is required. It is noted that a report of the teratology study was available in 1976, two years before issuance of tolerance on soybeans.

Review:

1) The Acute Oral Toxicity (LD_{50}) and the Neurotoxic Effects of CIPC on the Domestic Hen (Huntingdon Research Centre Report#PPG 4NT/80188; 6/5/80)

Test Material: CIPC technical

A. LD_{50} Study

Three groups of five hens each were administered either corn oil (control) or CIPC technical in corn oil at 5000 mg/kg.

Results: No deaths; LD_{50} > 5000 mg/kg

Food Consumption: Not recorded.

Body Weight: Control birds showed an overall increase; one test group showed a small overall decrease and the other test group showed a large increase in body weight.

Classification: Core-Minimum Data

B. Neurotoxicity Study

Groups of 10 birds were dosed with 1250, 2500 and 5000 mg/kg of CIPC. Ten birds were dosed with 500 mg/kg of TOCP which served as a positive control. Ten negative control birds were dosed with corn oil only.

Results: All negative control birds and birds dosed with CIPC had no mortalities and good health. All birds dosed with TOCP showed signs of ataxia. One bird was sacrificed on day 15.

Body Weight: The TOCP treated birds showed an overall decrease in body weight. All other groups showed an increase in body weight in the post-dosing observation period (21 days).

Food Consumption: Food consumption was variable in all groups.

Histopathology: Histologic lesions were observed in the TOCP treated birds which correlated with the ataxia observed. There was no histological lesions, considered treatment-related, in the CIPC treated birds.

Classification: Core-Minimum Data

2. Mutagenicity

1. Ames Assay (Study I) (Batelle; May 27, 1977)

Test Material: CIPC, Lot#447-408 in DMSO

The test strains used in this test were TA1535, TA1537 and TA98. The tests were conducted with and without metabolic activation by S-9 rat liver supernatant. Concentrations of 1, 10, 100 and 1000 ug/plate were tested. Positive controls were tested.

Results: The highest level of CIPC tested produced inhibition of bacterial growth. The following results were obtained:

	Strain	Conc./Plate	Toxicity: Ave. Colonies per plate	Toxicity: Relative Viability	Mutagenicity: Ave. Revertants per plate	Mutagenicity: Adj. Ave. No. of Rev.	Mutagenicity: Relative Mutagenicity
ctivation	TA-1535	1000	75.3	0.413	6.6	15.980	1.044
		100	117.6	0.645	13.3	20.620	1.347
		10	117.6	0.645	14.6	22.635	1.479
		1	126.3	0.692	11.0	15.895	1.038
		0	182.3	-----	15.3	------	-----
	TA-1537	1000	36.0	0.188	4.3	22.872	2.659*
		100	82.0	0.428	3.6	8.411	0.978*
		10	98.0	0.512	5.0	9.756	1.135
		1	126.6	0.661	5.6	8.472	0.985
		0	191.3	-----	8.6	------	-----
	TA-98	1000	77.3	0.287	29.3	102.090	2.736*
		100	137.0	0.509	33.3	65.442	1.753
		10	160.6	0.594	31.6	53.198	1.426
		1	219.0	0.814	34.6	42.506	1.139
		0	269.0	-----	37.3	-------	-----
onactivation	TA-1535	1000	79.3	0.523	10.0	19.120	1.405
		100	115.0	0.758	8.6	11.345	0.834
		10	91.0	0.600	9.0	15.000	1.102
		1	119.3	0.786	10.3	13.104	0.963
		0	151.6	-----	13.6	------	-----
	TA-1537	1000	42.0	0.199	4.6	23.115	3.502*
		100	133.6	0.634	3.6	5.678	0.860
		10	153.3	0.727	4.6	6.327	0.958
		1	134.3	0.637	4.6	7.221	1.094
		0	210.6	-----	6.6	------	-----
	TA-98	1000	46.0	0.383	18.6	48.563	1.471*
		100	77.3	0.644	21.3	33.074	1.002
		10	73.3	0.610	22.0	36.065	1.092
		1	116.0	0.966	24.0	34.844	0.752
		0	120.0	-----	33.0	------	-----

2. Ames Assay (Study II) (Batelle; March 10, 1978)

Test Material: CIPC; Lot#447-565 in DMSO

The test strains used were TA1537, TA100 and TA98. The tests were conducted with and without metabolic activation by S-9 rat liver supernatant. Concentrations tested were 1, 10, 100 and 1000 ug/plate. Positive controls were used. The following results were obtained:

		Activation					Nonactivation				
		Toxicity		Mutagenicity			Toxicity		Mutagenicity		
Tester Strain	Conc. ug/Plate	Plate Count	Rel. Via.	No. of Revts.	Adj. Revts.	Rel Mut.	Plate Count	Rel. Via.	No. of Revts.	Adj. Revts.	Rel. Mut.
TA-1537	1000	152	.84	3	4	0.4	33	.70	3	4	.8
	100	153	.84	6	7	0.7	32	.68	4	6	1.0
	10	149	.82	5	6	0.6	32	.68	4	6	1.0
	1	160	.88	9	10	1.0	39	.83	5	6	1.0
	0	182	---	10	--	---	47	---	6	-	---
TA-98	1000	166	.77	19	25	0.6	81	.76	13	17	0.7
	100	203	.93	27	29	0.7	67	.63	20	32	1.2
	10	189	.86	29	34	0.8	82	.77	28	36	1.4
	1	197	.90	42	47	1.1	107	1.00	28	28	1.1
	0	219	---	44	--	---	107	----	26	--	---
TA-100	1000	263	.75	130	173	1.3	223	.85	63	74	0.6
	100	274	.77	123	160	1.2	266	1.01	89	89	0.7
	10	280	.79	140	177	1.3	236	.90	98	109	0.8
	1	271	.77	137	180	1.4	267	1.02	117	117	0.9
	0	353	---	133	---	---	263	----	131	---	---

3. BALB/C 3T3 Transformation Bioassay (Battelle; March 10, 1978)

Test Material: CIPC; Lot#447-565 in DMSO

In a prescreen confluency cytotoxicity assay, concentrations of 1, 10, 50, and 100 ug/ml resulted in about 50% confluency. In the transformation study, concentrations of 0.1, 10, 70, 100, and 200 ug/ml produced the following results:

Concentration ug/ml	Ave. No. Colonies/Plate	Plating Efficiency	Transformed Colonies/Plate	Transformatic Frequency
200	0	0	0	0
100	52.8	13.2	0	0
70	154.4	38.6	0	0
10	147.4	36.75	0	0
0.1	80.6	20.15	0	0

Conclusions:

1. Ames Assay: Study I

   a. Strains TA1538 and TA100 were not tested.

   b. There were unexplained asterisks in Table 7 indicating values which appear to be positive (i.e., increased mutagenicity at higher levels).

2. Ames Assay: Study II

   a. Strains TA1538 and TA1535 were not tested.

   b. The dosages used were not high enough.

3. BALB/C 3T3 Transformatic. Assay

   a. There is not indication of positive controls.

   b. There is no indication of metabolic activation.

3. Discussion of the potential of CIPC to be an Inhibitor of Cholinesterase Activity; Company Abstract:

"Although CIPC structurally belongs in the general category of carbamates, it is in fact a carbanilate. Carbanilates have not demonstrated the anticholinesterase activity associated for example with N-methyl carbamates. Additionally, since the acute effects of CIPC are not indicative of a cholinergic response, it can be concluded that CIPC does not have anticholinesterase activity."

References

1. M.J. Kolbezen, R.L. Metcalf, and T.R. Fukuto; J. Ag. Food Chem. 2, 864-870, 1954

2. Herbicides; Chemical Degradation and Mode of Action; ed. Keamey and Kaufman; Vol. 2; pp. 609-664.

3. Vandekar et al., Bull. Wld. Hlth. Org. 1971, 44, 241-249

Conclusion: References were not submitted.

4. Teratology

According to the submitted material, "There are several uncertainties concerning the issue of hydrocephalus which make the significance of this reported finding questionable. The initial evaluation of the tissues was performed in a manner that allowed the possibility of an inconsistency in the scoring of tissues from the control group relative to the treatment groups. The incidence of hydrocephalus reported in the aspirin treated positive control group (16%) was much higher for this study than for the historical positive control data from Cannon Laboratories (2.7%), suggesting that this study differed from studies comprising the historical data from Cannon Laboratories either in scoring criteria or in spontaneous occurrence of moderate hydrocephaly and enlarged ventricles. The initial examination of the tissues from this study did not score for the severity of the hydrocephalus. An attempt was made by another laboratory to re-examine a portion of the tissues in order to grade them for the severity of hydrocephalus. No tissues from the negative control group were included in the specimens that were re-examined. Of the tissues re-examined, only 45% of those tissues identified as positive by Cannon Laboratories were confirmed by the second laboratory and this group also classified as positive 7% of the tissues which had been rated negative .

by Cannon Laboratories. These inconsistencies in scoring between the two laboratories combined with the possible alteration of the tissue samples due to storage and handling precluded a conclusive re-examination and scoring for the severity of hydrocephalus for all of the tissues. The findings from this second examination of tissues were partially incorporated into the Cannon Laboratories final report on this study as follows: the classification "moderate hydrocephaly" was used for those tissue classified as positive by both laboratories; the classification "enlarged ventricles" was used for those tissues classified as positive by Cannon Laboratories and negative by the second laboratory; those tissues identified as negative by Cannon Laboratories and positive by the second laboratory was reported as negative by Cannon Laboratories. These uncertainties concerning the initial scoring of the tissues, the possible alteration of samples during storage and handling, and the inconsistent scoring between the two laboratories make complete evaluation of the reported hydrocephalus impossible. In attempting to assess the significance of this finding, Dr. Clara Williams, a toxicology consultant for PPG Industries, discussed the findings with Dr. Wilson, the originator of the experimental technique in question. Based on Dr. Wilson's comments that a finding of moderate hydrocephalus in immature rat pups was common and not indicative of true hydrocephalus, Dr. Williams concluded that the report of moderate hydrocephalus in this study was not evidence for a teratogenic response caused by CIPC. Although Cannon Laboratories, Dr. Wilson and Dr. Williams have concluded that the reported hydrocephalus is not evidence for teratogenicity, uncertainties concerning this data make the Cannon Laboratories study inconclusive on the issue of hydrocephaly."

a. Investigation of Teratogenic and toxic potential of CIPC 40.4% Lot#237-1283 in Rats (Cannon Report#6E2322; October 4, 1976)

Test Material: CIPC 40.4% Lot#237-1283

Groups of 20 pregnant Sprague-Dawley rats were dosed with 0 (water), 250 mg/kg aspirin, 95 mg/kg, 950 mg/kg, and 4750 mg/kg of formulated suspension (corresponding to 38.4, 383.8 and 1919 mg/kg of technical) from days 6-15 of gestation. The dams were sacrificed on day 20. The parameters evaluated included the following: Behavioral reactions, dam body weight on days 0, 6, 11, 15 and 20, implantations, corpora lutea, number of live and dead fetuses, resorptions, fetal body weight, external, visceral and skeletal evaluation of fetuses.

Results: The body weight gain of 383.9 and 1919 mg/kg/day groups and the aspirin treated group was decreased in comparison to controls.

No deaths and no abnormal behavior was noted in the CIPC treated groups. There was no treatment-related necropsy in the CIPC treated groups. There were no treatment-related effects on implantations, resorptions, live and dead fetuses, and mean fetal body weight in the low and mid-dose CIPC treated groups. Fetal body weight was decreased in the high-dose CIPC treated group and aspirin group. The mean averages for these parameters is shown below:

	Implants	Corpora lutea	Viable Fetuses	Non-Viable Fetuses	Resorption	Average Fetal Wt.
Control	14.89	16.36	14.36	0.05	0.47	3.95
38.4	14.00	15.35	13.50	0.00	0.5	3.83
383.8	13.00	14.25	12.85	0.00	0.115	3.90
1919	13.85	14.35	13.50	0.00	0.025	3.55
Aspirin	13.70	16.20	13.00	0.004	0.65	3.08

External examination of fetuses showed 2 fetuses with umbilical hernia, 6 fetuses with exencephaly, and 1 fetus with spina bifida in the aspirin group.

The results of visceral and skeletal examination are presented in the following tables.

Visceral Examination

	LABORATORY CONTROL DATA	DOSE LEVELS mg/kg/day NEGATIVE CONTROL	38.4	383.8	1919	POSITIVE CONTROL 250	LABORATORY POSITIVE CONTROL DATA
Number of fetuses examined	602	85	87	86	85	81	410
Head -							
Enlarged cerebral ventricles	3	-	7	9	10	11	3
Hydrocephalus moderate	-	-	11[A]	8	6	2	8
Microphthalmia	-	-	-	-	-	1	1
Nasal cavity not opened	-	-	-	2	1	-	-
Trunk -							
Hydronephrosis slight	4	1	4	1	1	3	-
moderate	2	-	1	-	1	1	2
Lobe of lung absent	-	4	12	4	-	1	-
Lobe of lung abnormally small	-	1	1	1	-	-	-
Blood in stomach	-	4	6	-	-	-	-
Heart abnormally large	-	-	-	-	1	-	-
Heart abnormally small	-	-	1	-	-	-	-

A = Dam #37 - 4 of 5 pups displayed moderate hydrocephalus

	LABORATORY CONTROL DATA	DOSE LEVELS mg/kg/day NEGATIVE CONTROL	38.4	383.8	1919	POSITIVE CONTROL 250	LABORATORY POSITIVE CONTROL DATA
Abdomen -							
Ectopic kidney	-	-	-	-	2	1	1
Abdomen -							
Mottled surface of liver	-	-	-	-	2	-	-
Ectopic testis	-	1	-	-	-	-	-

Skeletal Examination

		DOSE LEVELS mg/kg/day					
	LABORATORY CONTROL DATA	NEGATIVE CONTROL	38.4	383.8	1919	POSITIVE CONTROL 250	LABORATORY POSITIVE CONTROL DATA
Number of fetuses examined	1,386	189	183	171	185	180	884
Abnormalities							
Sternebrae -							
Incomplete ossification	93	109	98	55	86	150	33
Non ossification	23	4	1	1	-	3	40
Cleft	3	14	7	2	4	32	33
Ribs -							
Wavy	5	-	1	1	-	-	13
Rudimentary 14th	112	94	103	96	109	77	97
14th pair	82	6	10	9	19	86	67
Vertebrae -							
Incomplete ossification	-	-	-	-	-	7	-
Non ossification	-	-	-	-	-	1	-
Cleft	15	12	16	20	18	42	21
Malaligned	-	-	-	-	-	1	-

It appears from the data that enlarged cerebral ventricles occur in the CIPC treated groups in a somewhat dose-related manner. This is a teratogenic effect, although the testing laboratory and the registrant argue differently. Hydrocephalus of the brain was also present but no dose-response effect was observed, although the incidence in the CIPC groups was higher than the control group and aspirin treated group. Although considered separately in the report, enlarged ventricles and hydrocephaly are considered the same type of terata. Also appearing with terata were fetuses with lobe of lung absent in the low-dose group. It is also noted that positive findings by the second lab were reported as negative in the report. These teratogenic effect are disputed by the laboratory and the registrant. Skeletal examination did not reveal any notable CIPC treatment-related effects.

Conclusions:

The study is positive for teratogenicity at all dose levels. The terata include enlarged cerebral ventricles, hydrocephalus and lobe of lung absent. The maternally toxic NOEL is the high-dose. The fetotoxic NOEL is the mid-dose with an effect of reduced body weight at the high-dose. The repeat teratology study by Wil-Laboratories did not show any indication of teratogenicity in Sprague-Dawley rats gavaged with technical CIPC. The teratology study in this report was conducted with CIPC 40.4% (Lot#237-1283). It is possible that the study was in error by Cannon Labs, as judged by the registrants, or that an inert ingredient of the unregistered formulation produced the terata. It is also noted that the incidence of visceral and skeletal findings in the control is greater than the incidence of these findings in historical control data. Also there does not appear to be a relationship between hydrocephalus and low fetal body weight.

Classification: Supplementary Data

a. The registrant is required to submit the Confidential Statement of formulation.

	LABORATORY CONTROL DATA	DOSE LEVELS mg/kg/day					
		NEGATIVE CONTROL	38.4	383.8	1919	POSITIVE CONTROL 250	LABORATORY POSITIVE CONTROL DATA
Incomplete ossification of							
1. Parietals	27	20	35	25	26	18	37
2. Interparietals	19	20	35	24	24	16	27
3. Occipitals	26	-	3	-	8	4	35
4. Frontals	19	10	19	11	16	11	37
Non ossification of							
1. Parietals	-	1	4	-	1	3	-
2. Interparietals	-	1	2	-	-	-	-
3. Occipitals	-	-	-	-	-	3	-
4. Frontals	-	1	-	-	-	3	-
OTHER -							
Fused radius and humerus	-	-	-	-	-	1	-

b. A Teratology Study in Rats with CIPC (WIL Research Labs Project No. WIL-81107; 1981)

Test Material: Technical CIPC; (PPG Lot No. 237-2778)

Groups of 25 pregnant Sprague-Dawley rats were orally gavaged with 0 (corn oil), 100, 350 or 1000 mg/kg/day of test material during days 6 to 19 of gestation. Clinical observations occurred daily. Body weight was recorded on gestation days 0, 6, 9, 12, 16 and 20. The dams were sacrificed on gestation day 20 and the number and location of viable and nonviable fetuses, corpora lutea, early and late resorptions, and implantations were counted. The spleen and liver of each dam was trimmed and weighed. All fetuses were weighed, sexed, and examined for external, skeletal and visceral malformations and variations.

Results:

Maternal toxicity, evidenced by clinical signs such as salivation, urongenital staining, and dried red material around the nares, mouth and eyes, was observed in the 350 and 1000 mg/kg/day groups. Body weight gains were significantly reduced in 350 and 1000 mg/kg/day groups. Body weight gain for the 100 mg/kg/day group was comparable to the controls.

There were no treatment-related effects on viable and non-viable fetuses, implantations, resorptions and fetal body weight.

Spleen weight of the 350 and 1000 mg/kg/day dams were significantly increased.

There were no treatment-relat[illegible]xternal skeletal or visceral malformations.

Fetuses at 1000 mg/kg/day [illegible]ncrease in rudimentary 14th ribs.

Conclusion:

Technical CIPC was not teratogenic at dosages up to 1000 mg/kg/day. The fetotoxic NOEL is 350 mg/kg/day. The maternal toxic NOEL is 100 mg/kg/day.

Classification: Core-Minimum Data

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