PC 237
TH-178

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

001783

4/19/82

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Ms. M. Mautz (16)
Registration Division (TS-767)

SUBJECT: EPA Reg.#100-599, 100-598, PP#8F2057, 9H5231, and FAP 8H5177 - Profenofos (CURACRON) on growing Cotton. CASWELL#266AA Acc. Nos.: 070513, 245718, 245717, 245716, 245715, 245710, 245709, 245720, 245719, 245721

Petitioner: Ciba-Geigy Corp.
Agricultural Division
Greensboro, North Carolina 27409

Action Requested:

Ciba-Geigy previously submitted mouse carcinogenic and two-year rat chronic feeding studies performed by IBT that subsequently were determined invalid.

The present August 20, 1981, submission contained repeated mouse carcinogenic, a 6-month dog, and two-year rat chronic feeding studies for our review, and also a teratology study for consideration in support of requested tolerances on cotton.

Recommendations by Toxicology Branch:

1. The requested tolerances for use of Curacron on or in cottonseed oil, eggs, meat including poultry, milk and dairy products are not toxicologically supported:

   a. The rabbit teratology study submitted 2/16/82 (Ciba-Geigy #785565) was classified Supplementary Data, and should be repeated. Data reporting was not adequate. The study design was not adequate.

   b. The rat teratology study submitted 2/28/80 (Ciba-Geigy #22741900) contained only summary data. Complete and adequate individual animal data should have been reported. This study was classified as Supplementary Data. Study deficiencies should be resolved, or the study should be repeated.

   c. An acceptable delayed neurotoxicity study should be submitted; however, the results of one neurotoxicity study designated Supplementary Data (#8580-10426) suggested that Curacron does not display delayed neurotoxic potential.

   d. Questions concerning the 3-generation reproduction study must be resolved or the study must be repeated.

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2. If deficiencies relative to the 3-generation reproduction and the rat teratology studies can be resolved, and if the petitioner agrees to submit repeated teratology and neurotoxicity studies within a reasonable length of time, the proposed tolerances can be supported.

3. The 6-month dog study cholinergic NOEL was used to establish an ADI; the dog study ChE effects were more sensitive than similar effects found in the 2-year rat chronic feeding study. A NOEL for the 6-month dog plasma and RBC Cholinesterase inhibition was tentatively determined to be 0.2 ppm (pending clarification of food analysis data). An ADI of 0.0005 mg/kg/day, and a MPI of 0.03 mg/day were established.

The total TMRC (0.0235 mg/day) utilizes 78.35% of the calculated MPI of 0.03 (see attached printout).

4. The results of a calculation to indicate the potential Curacron hazard to infant milk are 3.8 x the ADI. However, RCB memo of 2/14/79 (Donald Reed) shows that no Curacron residues will result from use. Therefore, no hazard to infant milk would result from the proposed use.

5. NOTE: The CSF states that Profenofos technical is 88% pure. Residue Chemistry Branch stated [redacted] Toxicology Branch considers that the toxicity studies designed to evaluate the active ingredient, Profenofos, also evaluated [redacted]

6. New Toxicity Data reviewed in the present report:

a) Teratology, rabbit, HDT = 30 mg/kg. Study is classified as Supplementary Data:

(1) Data reporting was not adequate, and;

(2) The study design was not adequate.

b) Six-month dog - Plasma/RBC ChE NOEL, tentatively 0.2 ppm (LDT). LEL = 2.0 ppm Core-Supplementary Data (pending clarification of feed analysis data).

c) Twenty-four month mouse oncogenicity. No oncogenic potential at levels as high as 100 ppm (HDT). Histopathologic NOEL for male and female mice = 100 ppm (HDT). Core-Minimum Data.

d) Two-year rat chronic feeding. Plasma and RBC ChE NOEL = 0.3 ppm. LEL = 10.0 ppm. Core-Minimum Data

e) An explanation for the extraordinarily high feed analysis data values at the 6th and 7th analyses should be provided (14-18/01/80, and 11-20/02/80). The analysis data for the 0.2 ppm nominal dose rate is critical, since it affects the ChE NOEL. (6 month dog study)

7. A valid 2-generation reproduction study is required.

A 3-generation reproduction study reviewed by Woodrow, 8/9/81 (IBT#623-07944), was later designated an invalid study during an IBT validation review by G. Burin, 4/8/80. This study was revalidated 3/19/82 by G. Burin, following receipt of additional information concerning the study. G. Burin reviewed the validated study 3/25/82 and classified the study as Supplementary Data:

a) The number of histopathological tissues examined could not be determined.

b) Animals selected for histopathology were not randomized.

c) Observations for the F_0 and F_1 generations were not recorded on a daily basis.

d) Animals dying during the study were not adequately examined histologically.

e) Raw data were not adequate: source, strain or age of animals; diet prep. records for weeks 1, 37, and 54; fewer diet analysis results than samples taken.

8. A valid neurotoxicity study is required. Two IBT neurotoxicity studies previously reviewed by Woodrow, 8/19/81, have recently been re-evaluated by the HPB Canadian group under a cooperative IBT validation effort.

a) HPB Canada designated (IBT#8580-11187) an invalid study.

b) IBT test #8580-10426 (Chicken Delayed Neurotoxicity) was designated "valid with reservations." Although there were questions regarding the study design and test compound (the EC formulation was tested), the available raw data did not indicate findings of delayed neurotoxicity.

IBT neurotoxicity study #8580-10426 was designated "valid with reservations" by HPB Canada which is equivalent to EPA's category of Supplementary Data.

9. A complete listing of Curacron IBT studies with current validation status is included at the end of this report.

Tolerances Requested:

The tolerances proposed for residues for the insecticide profenofos (trade name Curacron), 0-(4-bromo-2-chlorophenyl) 0-ethyl s-propyl phosphorothioate (aka CGA-15324) and its metabolites converted to 4-bromo-2-chlorophenyl (aka-55960) and calculated as 0-(4-bromo-2-chlorophenyl) 0-ethyl s-propyl phosphorothiate are as follows:

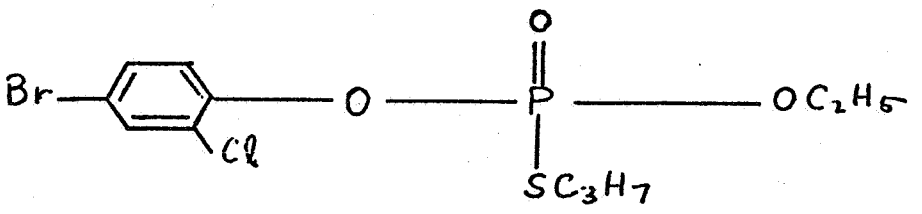
Commodity	Proposed tolerances (ppm)
PP8F2057, 9H5231, PP8H5177 (100-L00, 100-LOI)	
R.A.C.	
Cottonseed	3.0
Eggs and meat by-products of cattle, goats, hogs, horses, poultry and sheep	0.05
Milk	0.01
Processed Feeds	
Cottonseed hulls	6.0
Soapstock	15.0

A. Substance Identification:

1. Chemical Name: 0-(4-bromo-2-chlorophenyl) 0-ethyl s-propyl phosphorothioate (profenofos, CGA-15324)

2. Synonyms: Curacron, Curacron 6E, Curacron 6E A.I. = 59.6%

3. Structure:



B. Formulation

Curacron 6E Insecticide E.C.

Active Ingredient (CONFIDENTIAL)	Percent Weight
[redacted]	

Inerts cleared under 180.1001(c).

C. Previously Submitted Toxicity Data (From W. Woodrow memo, 8/19/81).

Acute Toxicity

Tech. Chemical - Ciba-Geigy No. 6021, Acc. No. 097794. Rat Oral LD_{50} = 334 mg/kg. Toxicity Category II, Core-Guideline Data.

Tech. Chemical - Ciba-Geigy No. 6020, Acc. No. 097794. Rat Oral LD_{50} = 344 mg/kg. Toxicity Category II, Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 6019, Acc. No. 097794. Rat Oral LD_{50} = 447 mg/kg. Toxicity Category II, Core-Guideline Data.

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Mouse Oral LD_{50} = 298 mg/kg. Toxicity Category II, Core-Guideline Data.

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Mouse Oral LD_{50} - 336 mg/kg. Toxicity Category II, Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rabbit Oral LD_{50} = 300 mg/kg. Toxicity Category II, Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Rabbit Oral LD_{50} = 700 mg/kg. Toxicity Category III, Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 5048, Acc. No. 097794. Rat I.P. LD_{50} = 585 mg/kg. Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rat Dermal LD_{50} = 1610 mg/kg. Toxicity Category II, Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rat Inhalation LC_{50} = > 2.15 mg/L air. Supplementary Data.

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Rat Inhalation LC_{50} = 3.00 mg/L air. Toxicity Category III, Core-Minimum Data.

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rabbit Primary Eye Irritation, P.I. Index = 0.2. Supplementary Data, Toxicity Category III

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rabbit Primary Skin Irritation, P.I. Index = 0.0. Supplementary Data

Subchronic and other Non-Chronic Toxicity Studies

Tech. Chemical - Ciba-Geigy No. 5119, Acc. No. 097797. Rabbit 21-Day Dermal. 5 of 6 rabbit (100 mg/kg) dead 1st week. No ChE NOEL Supplementary Data

Tech. Chemical - Ciba-Geigy No. 5119, Acc. No. 097797. Rat 21-Day Inhalation. No ChE NOEL. Supplementary Data

Tech. Chemical - Ciba-Geigy Test, Acc No. 097797 - Curacron tech. (CGA-15324) tested with methidathion or diazinon for potentiation. No potentiation. Core-Minimum Data

Tech. Chemical - Ciba-Geigy Test, Acc. No. 097797 - Antagonism by Atropine or Toxogonin. No antagonism of CGA-15324 activity. Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097797. Chicken Delayed Neurotoxicity. Acute Chicken Oral LD_{50} = 35 mg/kg. No delayed neurotoxicity. Supplementary Data

Tech. Chemical - Ciba-Geigy No. 31680, Acc. No. 097797. Salmonella/Mammalian Microsome Mutagenicity Test. Not mutagenic. Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 327438, Acc. No. 097797. Mouse Dominant Lethal Mutagenicity Study. Not mutagenic. Core-Minimum Data

Tech. Chemical 90-Day Rat Feeding Study (Hazelton #1519)
No NOEL (3 ppm LDT) determined for RBC or brain ChE activity.
Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 22741900, Acc. No. 097797. Rat Teratogenic Evaluation. Supplementary Data. Inadequate study design. Summary and inadequate reporting of data.

Tech. Chemical - Ciba-Geigy No. 3762, Acc. No. 097796. Dog 28-Day Feeding Study. No ChE NOEL. Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Guinea Pig Dermal Sensitization. No sensitization potential. Supplementary Data

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Guinea Pig Dermal Sensitization. No sensitizing potential. Core-Minimum Data

New Toxicity Studies Included in the Present Report

Tech. Chemical - Ciba-Geigy No. 785565. Rabbit Teratology Study. No terata reported (HDT = 30 mg/kg). Inadequate study design, inadequate reporting of data. Classification - Supplementary Data.

Tech. Chemical - Ciba-Geigy No. 790804. 6-Month Dog Toxicity. Plasma and RBC ChE NOEL = tentatively 0.2 ppm (LDT).
LEL = 2.0 ppm
Core-Supplementary Data (pending clarification of feed analysis data).

Tech. Chemical - Hazelton Laboratories No. 483-133. Twenty-four Month Mouse Carcinogenic Study. No oncogenic potential. Histopathologic NOEL for male and female mice = 100 ppm (HDT).
Classification - Core-Minimum Data

Tech. Chemical - Hazelton Laboratories No. 483-134. Two-Year Rat Chronic Feeding Study. Plasma and RBC ChE NOEL = 0.3 ppm.
LEL = 10 ppm
Classification - Core-Minimum Data

D. Review of New Toxicity Studies

Teratogenic potential of CGA-15324 Technical, Rabbits. Sponsor: Ciba-Geigy. Tester: Ciba-Geigy, Basle, Switerland. Project No. 785565. March 7, 1979.

Test Material

89.5% CGA-15324 technical in 2% aqueous carboxymethylcellulose (CMC), 4 ml of fluid/kg of body weight prepared by homogenizing, or using a magnetic stirrer.

Twenty virgin Chinchilla rabbits per vehicle control, and twenty/each of 5, 15 and 30 mg/kg test groups were mated by placing one male and one female in special breeding cages after 7 to 14 days acclimatization. Each doe was bred twice, the second time about one hour after the first time on the same day. This was considered day 0 of pregnancy.

The test material was administered orally by intubation from day 6 through day 18 of pregnancy. Control animals received a 2% aqueous solution of CMC.

Standard laboratory diet and tap water were available at all times throughout the experiment.

The general condition, weight gain and symptomology were checked daily. Food consumption was measured on days 6, 11, 15, 19, 24 and 28 of pregnancy.

Dams were killed by cervical dislocation and foetuses removed by Caesarean section on day 28 of pregnancy. Following assessment of the dam's organs, including the ovaries (corpora lutea counted) and uterus (mucosa and contents, including amniotic fluid and placentae as well as abortions and resorption sites) the foetuses were removed and subjected to careful external examination. They were then individually weighed and treated as follows:

1) Body cavities were examined (thorax, abdomen, pelvis), and sex were recorded;

2) The cephalic viscera were processed according to the slicing techniques of Wilson[1]: foetal heads were fixed in a mixture of trichloracetic acid and formol and preserved in ethyl alcohol.

[1]Wilson, J.G., in: Teratology, Principals and Techniques: J. Wilson and J. Warkany eds., The University of Chicago Press, Chicago and London, 1965, pp. 262-277.

3) An assessment of foetal trunks and limbs was made.

Data characteristic of the breed of rabbit used was presented (historical data).

Statistical evaluation of data was performed whenever feasible.

Results:

Maternal Data

No food consumption data was provided for review; figure 2 (graph) attempted to summarize the data, but is inadequate for review purposes. It appears from this graph that food consumption was less in the control than the test groups for the first 15 days of gestation.

The report indicated that "at sacrifice one dam of the 30 mg/kg dose group showed dysplasia and coalescence of uterus horns; no implantations were found." No additional necropsy findings of dams other than those in tables 1.1 to 1.4 (examination of uterine horns only) was available for review. No clinical observations data necessary for assessment of maternal toxicity was presented in the report.

No maternal body weights were included in the test report. A graph (Fig. 1) indicated body weight gain was reasonably consistent in all dose groups and controls during days 0-28 of gestation. In order to properly assess this parameter, however, data should be submitted.

One dam from the control, 5, and 30 mg/kg dose groups was found to have diarrhea prior to dosing and was excluded from the study. Additionally 5 dams from the control, 2 from the 5 mg group, 6 from the 15 mg group and 7 from the 30 mg group did not have any implantations and/or corpora lutea. Therefore, evaluation of teratogenicity in this study was limited to only 14 control dams, 17 from the 5 mg/kg group, 14 from the 15 mg/kg group and 12 from the 30 mg group.

Fetal Data:

Mean numbers of early and late resorptions per litter are presented below:

Dose Level	0	5 mg/kg	15 mg/kg	30 mg/kg
Early and late resorptions per total litters examined	11/14	11/17	15/14	12/12
# Resorptions per litter	.78	.64	1.07	1.0

By combining embryonic and fetal resorptions it is evident that there is a slight increase in the number of resorptions at 15 and 30 mg/kg. This is also evident (see below) when the data is evaluated as % resorptions similar to the methods used in the submitted report.

Dose Level	0	5 mg/kg	15 mg/kg	30 mg/kg
Total # implantations	148	154	122	106
Total # resorptions	11	11	15	12
% resorptions per total # implantations	7.4	7.1	12.3	11.3

Although variations in sex ratio were observed in the test groups, no significant compound related effects were evident.

The mean weights of the fetuses in each group are presented below:

Dose Level	0	5 mg/kg	15 mg/kg	30 mg/kg
Males (g)	33.9	33.8	35.8	37.0
Females (g)	32.9	34.2	35.6	38.1
Combined (g)	33.1	34.2	35.7	37.7

In both sexes of the 15 and 30 mg/kg dose groups there is an apparent increase in mean fetal weights. This increase is also evident when these weights are compared to the historical control value of 34.3 g for both males and females.

The following malformations were noted in the study (Tables 1.1-1.4):

Vehicle control (3 fetuses from 3 litters)

a) 1 fetus with multiple malformations including anencephaly, edema, omphalocele, ectromelia of fore-limbs, brachydactyly of the right rear paw (II & III) brachycaudia and lumbar kyphosis.

b) 1 fetus with generalized edema, arthrogryposis of right fore-limb.

c) 1 fetus with omphalocele, brachycaudia, caudal dystopia of left kidney, hypoplasia of right kidney.

In Table 6 of the report 3 fetuses were listed with skeletal malformations in the control group. These fetuses were identified as having either:

a) Dysplasia of the last sacral vertebrae (in association with brachycaudia)

b) Hemivertebrae 9-12 (thoracic) and dysplasia of the left scapula (in association with multiple malformation).

Unfortunately, as the data are presented only in summary form, it is impossible to know whether these skeletal malformations were identified in the same or different fetuses and litters than those listed in table 1.4.

Only one malformation (visible during external examination) was noted in the 5 mg/kg dose group (omphalocele) while none were noted in either the 15 or 30 mg/kg dose groups. No soft tissue or skeletal malformations were noted in any dose group. It is also noted, however, that no skeletal examination of the skulls was performed on any fetuses (since all heads were sliced) and that the reporting of soft tissue findings is limited only to the sliced heads (see table 5). The study is therefore considered unacceptable due to inadequate study design and also due to the summary manner in which the data is presented.

As an example of the inadequate presentation of the data, table 6 is noted. It is labeled "skeletal assessment" and attempts to present data on the extent of ossification and skeletal malformations found in the study. It is noted that (1) This data is only presented in summary form; (2) footnote "c" (sternum irregularly ossified) is not connected to the above table; (3) it is not clear from the table that only the trunks of the animals received a skeletal examination; (4) there are column headings for only the phalangeal nuclei and 5th sternebrae and it cannot be determined whether a complete skeletal examination of other ossification centers was in fact performed.

Conclusions

Available data does not indicate technical (89.5% a.i.) CGA-15324 to be teratogenic in the Chinchilla rabbit at doses as high as 30 mg/kg. Necessary data to evaluate maternal toxicity was not included in the test report. The NOEL for fetotoxicity appears to be 5 mg/kg.

Classification:

This study is classified as Supplementary Data due to:

1) Inadequate study design.

2) Summary and inadequate reporting of data.

6-Month Toxicity Study with Dogs, Using CGA-15324 Technical (Curacron) Sponsor: Ciba-Geigy. Tester: Ciba-Geigy Limited, Basle, Switzerland. Experimental Toxicology GU 2.1. Project No. 790804. June 17, 1981.

All in-life testing and necropsy was done at the Sisseln facility 14332, Stein, Switzerland. Pathological examinations were performed at the Rosental facility (4002 Basle, Switzerland).

Test Material

CGA-15324 technical, purity: 88.1%-89.3%. The technical chemical was weighed and diluted with PAG400 and then mixed with pulverized dog food and about 9% water to prepare dosage levels of 0.0, 0.2, 2.0, 100.0 or 500.0 ppm concentrations, which gave 0.005, 0.05, 2.5, or 12.5 mg/kg/day, respectively.

Dosage levels were pelleted and air dried. Pre-test and samples collected at various times during the test were analyzed for CGA-15324 test compound content. Dried pellets were stored at -20°C:

This single lot of stored, pelletized CGA-15324 (-20°C) was used throughout the study.

Groups of 7 male and 7 female Beagle dogs per dose were administered 0, 0.2, 2, 100, or 500 ppm in the diet daily for 182 days (26 weeks). One animal per sex and dose group was maintained on laboratory chow only for a 1 month posttreatment recovery period.

Prior to start of the test and at specified intervals, feed samples were analyzed for concentration and stability of the test material. Peleted standard diet at 350 g/day/animal was provided.

Observations

Mortality, signs and symptoms of local and/or systemic toxicity, and food consumption were recorded or measured daily. The body weight was measured weekly. Hematology and blood chemistry examinations were performed pretest, and during weeks 4, 9, 13, 18, 22 and 26; during week 31 for recovery animals.

Urinalyses were performed pretest, and during weeks 4, 9, 13, 18, 22 and 26; and during week 31 for recovery animals.

Ophthalmoscopy was performed pretest, and after 26 and 31 weeks.

Auditory perception was assessed weekly by simply calling each dog.

Mean food conversion (g food/kg body weight/day) was calculated using the following formula:

$$MFC = \frac{\text{Weekly food consumption (g)}}{\text{midweek body weight (g)}} \times \frac{1000}{7}$$

Laboratory Investigations

Blood sampling for hematology and blood chemistry was performed between the hours of 8 and 9 a.m., following withholding food for 20 hours.

Haematology parameters included: haemoglobin, erythrocytes, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, reticulocyte and thrombocyte counts, prothrombin time, partial thromboplastin time, thrombin time, leucocyte and differential counts.

Clinical chemistry studies included: glucose, urea, creatinine, total bilirubin, total protein, protein electrophoresis, asparagine aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, glutamyl transpeptidase, creatine kinase, cholesterol, acetyl cholinesterase (RBC, plasma, and brain), carboxylesterase, sodium, potassium, chloride, calcium, and phosphorus.

Urinalysis parameters: volume, color, specific gravity, pH, protein, glucose, ketone, blood, bilirubin, urobilinogin, and urine sediment.

Statistical Analysis

For each time point and parameter a single-variate statistical analysis was conducted. Due to the routine manner of the analysis system, parameter free methods were applied. Each treated group was compared to the control group with respect to dispersion and displacement. In addition a trend test was applied considering all groups.

Gross Pathology

At the end of the 6 month test period, or an additional 1 month recovery period, all treated and control dogs were bled under anaesthesia. The total weight of each animal was measured, and complete autopsies were performed. The following organs were weighed: heart, liver, kidneys, adrenals, thyroid, gonads and brain.

Histopathology

The brain (cerebrum, cerebellum, brainstem), spinal chord, eyes, pituitary, salivary gland, aorta, heart, thymus, thyroid, trachea, lungs with mainstem bronchi, spleen, lymph nodes (cervical and mesenteric), bone with marrow, esophagus, stomach, small and large intestine, liver, gall bladder, pancreas, adrenals, kidneys, urinary bladder, testicles, epididymis and prostate or uterus and ovaries, mammary area, skin, skeletal muscle and sciatic nerve were fixed in 10% buffered formalin and embedded for histological examination.

Neurologic Examination

A neurologic examination was performed once during treatment week 23 on all 7 male and 7 female dogs from the control group and from the top dose tested with CGA-15324 (500 ppm). These investigations included:

a) test for muscle force and muscle strength;
b) observation of motor coordination and gait, and;
c) testing of spinal and brain reflexes including visual and tactile response.

Results

Clinical Observations

No mortality or compound related clinical symptoms were observed. A transitory diarrhea occurred during the first two months of test in some dogs of all groups including controls, followed by a return to normal feces for the remainder of the experiment.

Eye Examinations

No treatment related findings were noted during eye examinations.

Auditory Perception

The dogs hearing was not impaired by treatment.

Food Consumption

The food intake of the treated animals was comparable to that of control animals, except for group 5 (500 ppm) male dogs, which consumed less food during the first three weeks of the study.

Body Weight Gain

The body weight gain of all animals was similar.

Food Utilization Efficiency

The mean food conversion ratio was not influenced by CGA-15324 treatment of male or female dogs.

TABLE I

Analysis of CGA-15324 in pelletized form; each analysis represents freshly prepared, pelletized food maintained at -20°C:

Analysis Number	No. Prep. Date (d/mo/yr.)	Mixed first fed date (d/mo/yr.)	Analysis data (d/mo/yr.)	PPM Nominal	PPM Analysis	%
1	?/08/79	?/?/79	17-20/09/79	0	<0.1	
				100	96	96
				500	471	95
2	?/?/?	10/?/79	10-14/09/79	0	<0.1	
				0.2	0.23/0.22	110
				2.0	1.46/1.5	73/?
3	04/10/79	06/10/79	15-19/10/79	0	<0.05	
				0.2	0.18	91
				2.0	1.6/1.7	80/86
				100	83/79	83/79
				500	390/420	78/84
4	?/?/79	07/11/79	3-6/12/79 (2-5 months)	0	<0.05	
				0.2	0.19	95
				2.0	2.14	107
				100	102	102
				500	520	104
5	28/11/79	04/12/79	10-14/12/79 (Approx. 3 months)	0	<0.05	
				0.2	0.23	114
				2.0	1.84	92
				100	102	102
				500	505	101

(Analysis of CGA-15324 in pelletized form continued)

Analysis Number	No. Prep. Date (d/mo/yr.)	Mixed first fed date (d/mo/yr.)	Analysis data (d/mo/yr.)	PPM Nominal	PPM Analysis	%
6	28/12/79	08/01/80	14-18/01/80 (5 months)	0	0.09	
				0.2	0.44-1.06	220/530
				2.0	1.57	79
				100	74	74
				500	377	76
7	18/01/80	23/01/80	11-20/02/80	0	0.25	
				0.2	0.98	
				2.0	3.98	
				100	70	70
				500	355	71
8	15/02/80	21/02/80	27-29/02/80 (5 months + 1 week)	0	0.05	
				0.2	0.166	80
				2.0	1.34	67
				100	80	80
				500	318	63.5

Study began ? 17-20 September, 1979,
Feeding ended February 25, 1980

? = illegible numbers

Several of the analytical values were far higher than nominally intended. For the sixth analyses (14-18/01/80), the intended dose rate was 0.2 ppm; analytically the value ranged from 0.44 to 1.06. Again during the 7th analysis (11-20/02/80), the analytical values for nominal 0, 0.2, and 2.0 ppm were 0.25, 0.98, and 3.98, respectively. Since the figures are all higher than nominal values, the errors are conservative and err on the side of safety; however, the tester should offer some explanation about why these figures are so high (the nominal 0.2 value is critical to this experiment for ChE NOEL effects).

Ophthalmic Examination

Eye examinations did not reveal any compound related effects.

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Auditory Perception

No impairment of auditory perception was found.

Laboratory Investigations:

a. Urinalysis

Urinalysis determinations for control and test animals were comparable.

b. Haematology and Blood Chemistry

The erythrocyte and haemoglobin concentration in males and females of the 500 ppm treatment group and the haematocrit in males of the 500 ppm groups did not increase with animal age as shown by controls and in the lower dose groups.

c. Cholinesterase (ChE) Activity

Dose (ppm)	Mean % ChE Inhibition			
	MALE		FEMALE	
Test Week 4	Plasma	RBC	Plasma	RBC
.2 ppm	9	0	0	0
2.0	47	0	45	0
100.0	77	52	77	66
Week 8				
.2	20	0	4	0
2.0	45	0	52	0
100.0	62	60	76	52
Week 13				
.2	17	0	2	0
2.0	54	2	64	0
100.0	74	61	79	35
Week 17				
.2	21	0	0	0
2.0	50	1	46	0
100.0	33	54	77	52
Week 21				
.2	5	0	2	0
2.0	36	16	47	1
100.0	75	72	70	80
Week 26				
.2	3	4	6	0
2.0	27	81	50	0
100.0	68	74	77	66
Week 30				
.2	0	0	5	0
2.0	0	24	0	0
100.0	25	65	0	0

Brain ChE activity was determined at 26 weeks of test on 6 male and 6 female dogs; one each from each dose level.

Males - the only brain ChE inhibition was 5.0% at the 2.0 ppm dose level;

Females - 8% brain ChE inhibition at the 0.2 ppm dose level, 10%, 11% and 5.0% brain ChE inhibition at the 2.0, 100.0, or 500.0 ppm dose levels, respectively.

Carboxylesterase activity of the liver was inhibited in the 100 and 500 ppm test groups of male dogs.

Summary of cholinesterase inhibition activity caused by dietary administration of CGA-15324 technical compound: An examination of the ChE activity data presented above indicates that a ChE NOEL of 0.2 ppm is apparent, based on plasma and RBC cholinesterase inhibition. The LEL = 2.0 ppm. One male and one female dog tested from each treated group showed some ability to regain ChE activity during a one month recovery period on laboratory chow only;

Plasma NOEL = 0.2 ppm
LEL = 2.0 ppm

RBC (M) NOEL = 0.2 ppm
LEL = 2.0 ppm

RBC (F) NOEL = 2.0 ppm
LEL = 100.0 ppm

Organ weights and ratios

The organ weights, organ to body weight and organ to brain weight ratios were within normal limits for the animals tested.

Pathology

No gross or histopathological changes in the organs or tissues were noted that could be related to treatment with CGA-15324 technical. All gross and histopathological lesions seen in some control and test animals were incidental in nature and were not test compound related.

Neurologic examination

Treatment with CGA-15324 in doses as high as 500 ppm failed to produce detectable signs of neurotoxicity.

Conclusions

The only significant adverse effect produced by CGA-15324 in male and female dogs during this 6 month dietary feeding study was inhibition of plasma and RBC cholinesterase activity at 2.0, 100.0, or 500.0 ppm doses.

A tentative NOEL for plasma and RBC cholinesterase inhibition was determined to 0.2 ppm, pending clarification of some feed analysis data, which showed extra high values (see Table 1 - this study).
LEL = 2.0 ppm.

Classification

Supplementary Data (pending clarification of feed analysis data).

Twenty-four Month Carcinogenicity Study in Mice with CGA-15324 (Curacron). Sponsor: Ciba-Geigy Corp. Tester: Hazleton Laboratories America, Inc. Project No. 483-133. July 23, 1981.

Test Material

Basal diet (Purina Rodent Laboratory Chow was used as the control material. The test material was CGA-15324, Batch No. FL77;423; 90.6% pure oily liquid.

The desired amount of test material (purity adjusted to 100% was added to a small amount of feed and premixed. The premix was then removed to a twin shell blender containing the remaining feed and mixed for one minute per kg of feed. Fresh diets were prepared and used weekly.

Aliquots of control and test diets were collected prior to initiation of the study and at each weekly mixing, frozen and shipped to the sponsor for analysis.

Animals

Five hundred ninety-nine (300 M and 299 F) weanling mice (HaM/ICR Swiss, Charles River CD-1) were obtained for the study. Animals were quarantined for 19 days.

Two hundred sixty mice/sex were selected and randomly housed in groups of five/sex. At start, body weights of males ranged from 19.8 to 34.4 g, and female weights from 15.2 to 26.4 g; animals were 41 days old at study initiation. Daily temperature and humidity readings were recorded.

Groups and Dietary Levels:

Group	No. Animals[a] Male	Female	Dietary Level (ppm)
1. (Control)	60 + 5	60 + 5	0
2. (T-I-low)	60 + 5	60 + 5	1
3. (T-II-mid)	60 + 5	60 + 5	30
4. (T-III-high)	60 + 5	60 + 5	100

[a]Five mice/sex/group were included for the twelve-month erythrocyte, plasma, and brain cholinesterase (ChE) determinations. These are the interim sacrifice animals.

NOTE: The tester did not state what the basis for selection of the 100 ppm high dose was; therefore, it is not known whether 100 ppm approached the MTD.

Observations

All animals were observed at least once daily for mortality and morbidity. Individual body weights and clinical observations were recorded every four weeks. Food consumption was measured and recorded once every four weeks for each animal cage. Animals were palpated monthly for the first forty-one weeks of study and weekly thereafter for the presence of nodules, tissue masses, and wart-like lesions.

Clinical Laboratory Studies

Erythrocyte, plasma, and brain ChE determinations were performed on five animals per sex per group at week 53, on six male animals from Group 1 and 2 and five male animals from Groups 3 and 4 at week 85, and on ten female animals per group at week 97. Blood samples were collected from the orbital sinus at weeks 53 and 85 and from the abdominal aorta at week 97.

NOTE: The original study design called for a 104-week treatment period (24 months); however, survival reached 20% of the original number of animals in the mid-dose males and in the high-dose females, therefore males were sacrificed at week 85 and females were sacrificed during week 97. The study was initiated on March 13, 1978, and was terminated on October 24, 1979 (males) and January 16, 1980 (females).

Sacrifice and Gross Pathology

Five animals per sex group were sacrificed by design at week 53. At termination (weeks 85 and 97 for males and females, respectively), all surviving animals were weighed and sacrified. Gross necropsies were performed on all sacrificed animals at weeks 53, 85, and 97 on all mice found dead and sacrificed moribund.

Tissue Preparation

The following tissues from each necropsied animal were preserved in 10% buffered formalin: brain, pituitary, spinal chord, eyes with optic nerves, submaxillary salivary glands, trachea, esophagus, lungs, heart, aorta, liver, spleen, kidneys, adrenals, stomach, pancreas, small intestine (duodenum, jejunum, ileum), cecum colon, cervical lymph nodes, mesenteric lymph nodes, urinary bladder, testes or ovaries, prostate or uterus, skin with mammary gland, femoral or stromal bone marrow, nerve with muscle, and unusual lesions.

Histopathology

All of the preserved tissues from all control and high-dose animals were embedded in Paraplast, sectioned and stained with H&E, and examined microscopically. Organs from the mid and low dose groups that appeared abnormal at time of necropsy were also histopathologically examined.

Statistical Analysis

Individual rates of body weight gain were compiled using body weight values from the following intervals: males weeks 0, 4, 8, 12, 20, and 52 and females weeks 0, 4, 8, 16, 24, and 52. Food consumption values were compiled from data obtained at the intervals above for body weight gain. The growth rate and food consumption of the control group were compared to the data of the treated groups of the same sex by Bartlett's test for homogeneity of variance. This analysis was followed by a one-way classification analysis of variance (ANOVA). Clinical laboratory data of the control group were compared statistically to the data of the treated groups of the same sex by Bartlett's test for homogeneity of variance. This analysis was followed by a one-way classification analysis of variance (ANOVA). The mean body weight values at weeks 0 and 52 of the control group were also compared to those of the treated groups by a multiple pairwise comparison procedure. Survival through weeks 85 (male) and 96 (females) was analyzed by a life table technique. All analyses were evaluated at the 5.0% probability level.

Results:

Mortality and Clinical Observations:

Mortality:

Group	Dietary Dose (ppm)	21 Months (85 Weeks) Male - No. Dead/ Total[a]	% Survival	24 Months (96 Weeks) Female - No. Dead/ Total[a]	% Survival
1 (control)	0	38/60 + 5	37	39/60 + 5	35
2 (T-I-low)	1	33/60 + 5	45	42/60 + 5	30
3 (T-II-mid)	30	39/60 + 5	35	45/60 + 5	25
4 (T-III-high)	100	38/60 + 5	37	47/60 + 5	22

[a]Interim sacrifice animals included.

These (mortality) data represent all animals that died or were sacrificed in a moribund condition, and those animals found dead throughout the study. These figures exclude the interim sacrifice animals (mortality), and also exclude:

4 Group 1 and 9 Group 3 males and 1 Group 1 female which died due to automatic watering and malfunction;

1 Group 3 male and 1 Group 4 male, which were missing;

2 Group 4 females - 1 was accidentally drowned and the other accidentally killed.

Statistical analysis of survival data for male and female animals revealed no significant differences between control and treated animals.

No compound-related clinical signs were noted in any animals.

Diagnostic microbiology was performed on blood and/or tissues during weeks 73 and 76 from one Group 2 male, two Group 2 females, and one Group 4 male. Cultures produced no growth except for Escherichia coli on one liver sample (normal gut flora).

Tumor Incidence and Gross Pathology

No treatment-related differences were noted in the incidence of palpable nodules, tissue masses, and wart-like lesions between control and treatment groups.

No distinct treatment-related organ or tissue alterations were noted at necropsy in any of the animals. Tissue and organ changes that were observed were stated to be common to the strain and age of mice used in the study. Frequently observed incidental findings included generalized amyloidosis (most consistently noted in the adrenals, thyroids, heart, liver, spleen, kidneys, stomach, salivary glands, small intestines, and ovaries); mononuclear cell infiltration in the lung, liver, and kidneys, and pigmented macrophages in the adrenals of the males were also frequently observed.

Body Weight and Food Consumption

Statistical analysis of individual growth weights and food consumption values for males and females during the first 52 weeks of the study revealed statistically significant differences that were not dose-related. Mean body weights for males and females at weeks 0 and 52 showed a significantly increased mid-dose value for females and a significantly decreased value for high-dose females at week 0; however, there were no significant differences found for females at week 52 or for males at either interval. Food consumption data revealed significantly increased values for mid- and high-dose males and for low-, and mid-, and high-dose females at week 0.

Mean body weights and food consumption values from weeks 56 through termination were comparable between control and treated groups.

Compound Consumption

Group mean compound consumption; weeks 1-84 - males, weeks 1-96-females:

Group	Dosage Level (ppm)	mg/kg/day Males	Females
1 (control)	0	0.0	0.0
2 (T-I)	1	0.1421	0.1911
3 (T-II)	30	4.5442	5.7736
4 (T-III)	100	14.2427	19.1857

Clinical Laboratory Studies

Treatment-related inhibition of erythrocyte and plasma cholinesterase activity was shown for both male and female mice from the mid- and high-dose groups at week 53 and at the termination of the study. No apparent depression plasma and erythrocyte cholinesterase activity for male and female mice at week 53 and at termination for the low dose level (1 ppm) was noted. Mid- and high-dose level treatment resulted in ChE inhibition greater than 20% for both male and female mice at week 53, and at termination (plasma and RBC cholinesterase).

Histopathology

Gross pathological findings were consistently accompanied by histopathology reports. No treatment-related findings were apparent when the incidence and types of neoplasms for control and high-dose (100 ppm) male mice were compared. However, when totals of benign and malignant neoplasms occurring in 60 control and 60 high-dose treated females were compared, 47 were found in control animals and 79 in high-dose females.

Examination of high-dose female individual histopathology records revealed that the increased incidence of neoplasms was due to widespread occurrence of malignant lymphomas occurring in only a few animals. When malignant lymphomas occurring in high-dose females were counted as 1 per affected animal, the incidence of total numbers of neoplasms in control and high-dose female mice was comparable:

Five female mice in the control and high dose (100 ppm) test groups displayed malignant lymphomas:

Group	Animal Number	Number Malignant Lymphomas
1 (0 ppm)	79047	17
	79061	1
	79070	21
	79075	5
	79088	1
		45 Total
4 (100 ppm)	79425	12
	79432	16
	79434	14
	79435	3
	79441	21
		66 Total

Thus, 5 female mice in the high dose (100 ppm) test group had 21 more malignant lymphomas than did 5 female mice with malignant lymphomas in the control (0-ppm) group. Since each mouse with malignant lymphomas may be considered to have 1 such neoplasm, the following calculation may be made to allow a comparison between control and high dose female test animals that showed malignant lymphomas;

79 = total number of benign and malignant neoplasms in the high
-21 test group.
58 total number of benign and malignant neoplasms - high test group accounting for higher numbers of malignant lymphomas.

Therefore 58 (benign and malignant neoplasms for the high test group), is comparable to 47; total benign and malignant neoplasms for the control group.

Conclusions:

Thus, no treatment related histopathological findings were noted, and CGA-15324 technical was not determined to be oncogenic in the present study.

A ChE NOEL for erythrocyte and plasma cholinesterase inhibition was determined to be 1 ppm for male and female mice.

A histopathologic NOEL was determined to be 100 ppm (HDT) for male and female mice in the present study.

Classification

Core-Minimum Data

Two-Year Chronic Oral Toxicity Study in Albino Rats with CGA-15324 Technical (Curacron). Sponsor: Ciba-Geigy. Tester: Hazleton Laboratories America, Inc. May 22, 1981. Project No. 483-134.

Test Material

Technical CGA-15324, 90.6% pure was incorporated into the basal lab. diet on a weight (active)/weight basal diet basis and mixed in a twin-shell blender (the purity of the test material was corrected to 100 percent purity when preparing test diets). Control rats received basal diet only. Fresh diets were prepared and offered weekly throughout the study. Samples of each weekly diet mixing were collected for analysis.

Five hundred ninety-nine (300 M and 299 F) Fisher 344 rats were obtained from the Charles River Breeding Labs. Following three weeks laboratory acclimation, two hundred sixty rats of each sex were randomly assigned to treatment groups as follows:

Group	Number Animals Male	Female	Dose Level (ppm)
1 (control)	60+5[a]+5[b]	60+5[a]+5[b]	0
2 (T-I)	60	60	0.3
3 (T-II)	60	60	10.0
4 (T-III)	60+5[a]+5[b]	60+5[a]+5[b]	100.0

a = Number of animals sacrificed at 12 months. (52 weeks)
b = Recovery group animals. Recovery group animals were continued on basal diet only for 11 additional weeks, and then sacrificed (week 63).

All animals on test were observed at least once daily for morbidity and mortality. Body weights, food consumption, and clinical signs for each animal were recorded weekly for the first fourteen weeks and monthly thereafter. Examination and palpitation of each animal for incidence and location of tissue masses were performed weekly and the observations recorded (from week 19 onward).

Clinical Laboratory Studies

hematology -
- hematocrit
- hemoglobin
- prothrombin time
- activated partial thromboplastin time
- erythrocyte counts
- platelet counts
- differential and total leukocyte counts

clinical chemistry-
- serum glutamic oxaloacetic transaminase
- serum glutamic pyruvic transaminase
- blood urea nitrogen
- alkaline phosphatase
- fasting blood glucose
- total cholesterol
- total protein

urinalysis-
- albumin
- glucose
- ketones
- bilirubin
- pH
- specific gravity
- microscopic element examination

Plasma, erythrocyte, and brain cholinesterase determinations were conducted at selected intervals:

Test	Interval(s)	Groups	Rats/Sex[a]
Clinical Laboratory Studies (Hematology, Clinical Chemistry, Urinalysis):	Weeks 13, 26	1, 4	10
	Weeks 52, 78, 105	1, 2, 3, 4	10[b]
	Week 63	1, 4 (recovery animals)	5[c]
Plasma and Erythrocyte Cholinesterase	Weeks 13, 26, 52	1, 2, 3, 4	10
	Weeks 57 (repeat samples during Week 58), 78, 105	1, 4 (recovery animals)	5
Brain Cholinesterase	Week 52	1, 4	5
	Week 105	1, 2, 3, 4	10[b]

[a]The method of selection was to choose in sequence from the first animal onward in each group tested.

[b]With the exception of Week 105, samples from twelve animals were analyzed in low-dose males and high-dose females and from eleven animals in mid-dose males for hematology, clinical chemistry, urinalysis, and blood and brain cholinesterase values to insure that at least ten samples were analyzed under every parameter.

After receiving the test diets for fifty-two weeks, the predesignated recovery animals were placed on the control diet and after four weeks recovery studies were done. The animals were sacrificed at Week 63, and until sacrifice they were fed control diet.

Blood samples from hematological determinations except prothrombin and activated partial thromboplastin time were collected by tail segmental amputation at all intervals except week 105 when samples were obtained from the abdominal aorta during terminal sacrifice. Blood samples for clinical chemistry determinations, prothrombin and activated partial thromboplastin time were obtained by orbital sinus puncture at weeks 13, 26, 53 and 78, and from the abdominal aorta at week 105. Urine samples were collected by housing rats in metabolism cages.

Sacrifice and Gross Pathology

Five rats/sex in the control and high dose groups were sacrificed at week 52 (interim sacrifice) 5 rats/sex in the control and high dose groups were sacrificed at week 63 (recovery animals), and all survivors were sacrificed between weeks 104 and 106. Necropsies were performed and gross observations recorded. Necropsies were also performed on rats that died or were sacrificed in extremis.

Organ Weights

At the interim sacrifice, recovery and terminal sacrifice, the following organs were weighed and organ/body weight, and organ/brain weight ratios determined: prior to fixation - testes with epididymis, brain, heart, liver, spleen, and kidneys; after fixation - thyroid, adrenals and ovaries with fallopian tubes.

Tissue Preservation and Histopathology

The following tissues were taken for histopathological examination from each sacrificed rat, rats sacrificed in extremis, rats that died, and fixed in 10% neutral buffered formalin: brain, pituitary, spinal chord, eye, optic nerve, salivary gland, thyroid, parathyroid, trachea, esophagus, lung, heart, aorta, liver, spleen, kidney, adrenal, stomach, pancreas, small intestine (duodenum jejunum, ileum), large intestine (colon, cecum), cervical and mesenteric lymph nodes, urinary bladder, testes with epididymis, prostate, seminal vesicle, ovary, uterus, skin, mammary gland, bone marrow (femur), sciatic nerve, muscle, Harderian gland, and any unusual lesions. Additional histopathology was performed on organs with tumors and lesions in low and mid-dose animals indicated by pathology findings in the high-dose group.

Statistical Analyses

The individual rates of body weight gain were determined by the method of Rao. Individual growth rates, total food consumption values, clinical laboratory data, and organ weight and ratio data of the treated groups (low, mid- and high-dose) were compared statistically to the control group of the same sex by Bartlett's test for homogeneity of variance and by the one-way classification analysis of variance (ANOVA). Clinical laboratory data and organ weight and ratio data of the control group for week 105 were compared to the data of the treated groups of the same sex by Bartlett's test for homogeneity of variance. In those instances where data were collected from the control and high-dose groups only, the statistical procedure used was ANOVA. The body weight changes and the food consumption values of the recovery animals were also analyzed by ANOVA. Survival through week 104 was analyzed by a life table technique (Sachs, 1959).

All analyses were evaluated at the 5% probability level.

Results

1. Mean % Survival

		Per cent Survival			
Group		1	2	3	4
Dose (ppm)		0	0.3	10	100
54 Weeks	M	83	82	82	89
	F	99	99	99	88
104 Weeks	M	85	90	80	83
	F	77	72	78	68

There were no statistically significant differences between survival groups of control and treated rats at 54 or 104 weeks of test.

2. Body Weights - Were unaffected by treatment with CGA-15324 at all dose levels.

3. Food consumption - Rats throughout the 104 week treatment period were unaffected by treatment at all dosage levels.

4. Clinical symptoms - Observations of CGA-15324 treated and control rats did not reveal any compound related differences in clinical signs.

5. Hematological Studies - No distinct compound-related trends in hematology values were evident in the treated groups of rats when compared to control animals, although some values were statistically significantly higher or lower than comparable control values. All of the hematology findings that represented statistically significant differences between control and treatment animals, however, were within acceptable laboratory limits.

The hematocrit and hemoglobin group mean values for high dose (100 ppm) male rats at weeks 52 and 78 were slightly lower than control values to a statistically significant degree; however, by week 105, these values had returned to normal.

A non-dose related, inconsistent, slightly lowered RBC group mean count at weeks 26 and 52, and WBC counts at week 13 was also observed in high dose male rats. These cited values were lower than comparable control values (statistically significant); all values returned to normal in subsequent assays. These effects were only seen in one instance in female rats; at week 26 the group mean value for 100 ppm females was slightly lower than controls.

Platelet counts and prothrombin time group mean values for test animals were comparable to control values at all dose levels.

Statistically significant (p = 0.05) lowered activated partial thromboplastin group mean time in male rats was observed at all three dose levels (0.3, 10, and 100 ppm), only at the terminal week, 105. Female rats showed statistically significant lowered activated partial thromboplastin times at all 3 dose levels at week 52, and at weeks 13, 26 (high dose only tested), and at week 105 only at the high dose level.

However, in the instances where activated thromboplastin times were statistically significantly lower than untreated control values, the standard deviations for control and test values varied so much that control and test activated partial thromboplastin time values theoretically overlapped in all but two individual instances:

Group and Dose Level ppm		Activated Partial Thromboplastin Time (sec) Week				
		13[b]	26[b]	52	78	105
				Males		
1	Mean	40.22	33.82	30.12	27.18	37.6
0	S.D.	10.45	6.77	10.04	6.99	7.14
2	Mean			25.56	24.91	28.3[S-]
0.3	S.D.			6.66	5.91	1.47
3	Mean			26.77	24.71	27.9[S-]
10	S.D.			2.84	5.04	4.21
4	Mean	34.23	32.70	21.69	30.39	25.4[S-]
100	S.D.	7.54	10.16	3.50	8.07	3.10

[a]Mean based on 10 animals/sex/group.
[b]Study was conducted only on Groups 1 and 4 animals at Weeks 13 and 26.
S- = Statistically significant p = 0.05

Group and Dose Level ppm		Activated Partial Thromboplastin Time (sec) Week 13[b]	26[b]	52	78	105
				Females		
1	Mean	40.65	30.48	37.11	23.30	32.9
0	S.D.	7.81	8.45	6.38	5.81	4.41
2	Mean			26.13^{S-}	22.44	32.7
0.3	S.D.			4.55	3.57	3.07
3	Mean			27.15^{S-}	21.65	31.9
10	S.D.			5.56	1.42	4.38
4	Mean	31.33^{S-}	22.04^{S-}	22.86^{S-}	22.11	25.9^{S-}
100	S.D.	10.35	4.18	1.13	3.25	2.88

[a]Mean based on 10 animals/sex/group.
[b]Study was conducted only on Groups 1 and 4 animals at Weeks 13 and 26.
S^- = Statistically significant (lower) p = 0.05

A dose related effect of test material v.s. activated partial thromboplastin time was not apparent.

A shortened activated partial thromboplastin time does not have a toxicological significance; instead, it indicates very poor test technology. The thromboplastin assays require strict adherence to exacting protocols, and any slight deviation will produce widely varying results.

Therefore, the statistically significant lowered activated partial thromboplastin times shown above are considered not treatment related, and quite probably were due to poor laboratory test execution.

Although some hematology parameters were statistically significantly less than control values, the incidence was inconsistent and not dose related within groups of animals of the same sex, or between male and female treatment groups.

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6. Clinical Chemistry Studies

TABLE I

Mean RBC Cholinesterase % Inhibition (pH/min)[a]

Males	0.3	5.7	-5.9	-5.3	-4.7	-4.5
	10.0	31.1	25.7	23.1	22.2	11.9
	100.0	66.8	67.2	69.4	70.6	58.2
Females	0.3	4.8	-9.8	7.7	6.2	10.0
	10.0	31.3	16.3	23.4	21.6	18.6
	100.0	64.0	60.9	66.3	60.7	58.6

TABLE 2

Mean Plasma Cholinesterase % Inhibition (pH/min)[a]

		Weeks				
	Dose (ppm)	13	26	52	78	105
Males	0.3	12.0	-23.2	1.9	14.8	17.9
	10.0	27.9	-8.6	15.2	23.0	23.1
	100.0	30.4	35.3	37.7	49.7	62.4
Females	0.3	2.5	-6.9	0.0	1.2	-2.3
	10.0	24.3	13.6	9.2	13.7	25.2
	100.0	57.9	58.1	49.6	54.0	62.4

TABLE 3

Mean Brain Cholinesterase % Inhibition (pH/min)[a]

		Weeks	
	Dose (ppm)	52	105
Males	0.3		1.4
	10.0		1.4
	100.0	-1.9	4.2
Females	0.3		5.7
	10.0		8.9
	100.0	6.3	11.5

[a] $\frac{\text{Mean Control Value - Mean Treated Value X100}}{\text{Mean Control Value}}$

Summary ChE effects:

The NOEL for erythrocyte (RBC) and Plasma Cholinesterase (ChE) inhibition for male and female rats was a dietary level of 0.3 ppm (LDT).

The LEL was the next highest dose tested; 10.0 ppm.

Some of the remaining clinical chemistry values were statistically significant; however, no dose related effects were apparent, and these data are considered of no biological significance. Significantly lower than control mean values included: fasting glucose level - Group 4 females at Week 26; serum glutamic pyruvic transaminase level - Group 2 males at Week 52 and Group 4 males at Week 26; total protein - Group 4 males at Week 26.

7. Urinalyses - Urinalyses at weeks 13, 26, 52, 78 and week 105 were unremarkable.

8. Gross Pathology - No compound related gross pathological findings were found in any of the interim sacrifice animals, the recovery animals, the terminal sacrifice animals, or the animals that died or were sacrificed in a moribund condition.

9. Organ Weights - The absolute and relative organ weights, and brain/organ weight ratios of the interim sacrifices, recovery animals, and terminal sacrifices were similar; some isolated statistically significant effects were noted; however, these were apparently of no biological significance: A significantly higher mean relative to body weight thyroid weight in Group 4 males compared to the control was found.

Gross pathological examination of Group 4 (100 ppm) male thyroids revealed 1 pale and 1 enlarged thyroid gland. Histopathological exarination revealed parafollicular cell adenoma and parafollicular cell carcinoma of the thyroid; observed in survivors and non-survivors, and group prevalences were similar in both sexes of treated and untreated rats. Follicular adenomas of the thyroid were observed in Group 3 male survivors, a Group 1 female survivor, and a Group 2 female survivor. Follicular carcinoma of the thyroids were observed in a Group 1 male survivor and a Group 3 female survivor. Thus, gross and histopathological analysis of thyroids revealed that Group prevalences of lesions were similar in both sexes of control and treated rats.

For the recovery animals, a statistically significant higher than control mean absolute adrenal weight was noted in Group 4 males, and a statistically significant lower than control mean relative to body weight ovary weight was noted in the Group 4 females. Analysis of absolute and relative organ weights at terminal sacrifices revealed comparable values for all treated and control groups.

10. Histopathology - No compound-related histomorphologic alterations were noted during microscopic examinations including neoplastic and non-neoplastic lesions of the: cardiovascular, respiratory, digestive, endocrine, urinary, male and female reproductive, the lymphoreticular, or central nervous systems, or mammary glands. A similar frequency and severity of commonly seen spontaneous disease lesions and incidental findings were observed in the surviving control and experimental rats of both sexes after 104 weeks of treatment.

Conclusions

CGA-15324 (Curacron technical) fed in the diet to male and female rats did not produce any significant or dose related mortality, clinical effects, alterations in body weight or food consumption, or palpable masses or tumors, or significant alterations in urine content or chemistry. The only significant (> 20%) effects noted were RBC and plasma ChE inhibition at a diet level of 10 ppm (LEL). The RBC and plasma ChE NOEL for male and female rats was 0.3 ppm.

Classification

Core-Minimum Data

f. Evaluation of Requested Tolerances:

Calculation of ADI using a NOEL determined for the 6-month dog study, submitted August 20, 1981:

The previously submitted IBT studies (90-day dog, mouse chronic feeding, or 2-year rat chronic feeding) have been evaluated as invalid and thus may not be used for establishing an ADI.

The present submission included a repeated 2-year rat chronic feeding study, and a 6-month dog chronic toxicity study. Both of these studies were classified as Core-Minimum Data, and may be considered for establishing an ADI for Curacron.

NOEL for Plasma and RBC ChE inhibition is 0.3 ppm for the 2-year rat study:

1 ppm = 0.050 mg/kg/day
0.3 ppm = 0.015 mg/kg/day

NOEL for Plasma and RBC ChE inhibition is 0.2 ppm for the 6-month dog study which represents the lowest figure, or the most sensitive animal, established in these two studies. Brain ChE activity was relatively unaffected by treatment in both the 2-year rat and 6-month dog studies.

1 ppm = 0.025 mg/kg/day

0.2 ppm = 0.005 mg/kg/day

$\frac{0.005 \text{ mg/kg/day}}{10 \text{ (S.F.)}} = 0.0005 \text{ mg/kg/day} = \text{ADI}$

MPI = 0.0005 x 60 kg = 0.03 mg/day

Conclusion (Evaluation of Requested Tolerances)

Pending approval of requested tolerances the total TMRC (0.0235 mg/day, 1.5 kg) utilizes 78.35% of the calculated MPI of 0.03 (see computer printout).

Estimated hazard to infants consuming milk from cows potentially exposed to Curacron residues:

Calculation - $\frac{0.77 \text{ kg x tolerance (mg/kg)}}{4 \text{ kg}}$

(Result should not exceed the ADI)

Therefore, $\frac{0.77 \text{ kg x } 0.01 \text{ (mg/kg)}}{4 \text{ kg}} = 0.00192$, ADI 0.0005. Thus, $\frac{.00192}{.0005} = 3.84$.
The infant exposure calculation exceeds the ADI by a factor of 3.84.

However; according to RCB March 23, 1979 memo (Donald Reed), no Profenofos residues are likely to occur in milk. Thus, the proposed use of Curacron on cotton is not likely to adversely affect human infants.

NOTE: The studies listed below considered pivotal for decision making are:

a. IBT#623-07924; 3-generation reproduction study. This study was validated and revalidated on April 8, 1980, and March 19, 1982, by Gary Burin. Gary Burin reviewed the validated study 3/25/82 and classified the study as Supplementary Data.

b. IBT#8580-11187; Delayed Neurotoxicity. This study was declared invalid by HPB Canada.

c. IBT#8580-10426; Delayed Neurotoxicity. HPB Canada designated this study "valid with reservations". Toxicology Branch classified it as Supplementary Data.

Complete list of Curacron IBT Studies (a copy of this list is to be included in the Caswell file):

1. Technical Chemical, Three-generation reproduction. IBT#623-07924. This study was validated and revalidated, following receipt of additional information concerning the study from Ciba-Geigy, on April 8, 1980, and March 19, 1982, by Gary Burin. Gary Burin reviewed the validated study 3/25/82 and classified the study as Supplementary Data based on the following deficiencies and discrepancies:

a) The number of tissues examined microscopically could not be determined.

b) A bias was introduced into selection of animals for histopathology due to animals being selected in a nonrandom manner.

c) Observations were not recorded on a daily basis for the F_0 and F_1 generations.

d) Animals dying during the course of the study were not examined histologically to the extent required by the protocol.

e) Other deficiencies and discrepancies were concerned with the lack of raw data for environmental conditions, source, strain or age of animals, diet prep. records for weeks 1, 37 and 54 and fewer diet analysis results than samples taken.

2. Formulation, Neurotoxicity - IBT#8580-10426, 2/17/80.

Birds treated with 38% a.i. formulation. Two 21-day successive treatments - doses of 44.5 mg/kg a.i. No neurotoxic signs, or histological evidence of delayed neurotoxicity.

HPB Canada designated this study "valid with reservations." Toxicology Branch classified it Core Supplementary Data.

3. Technical Chemical. Neurotoxicity - IBT#8580-11187. Invalid (HPB Canada).

4. Technical Chemical. 90-day dog - IBT#611-05912-A. Dog Subacute Oral Feeding "Final Report" - previously reviewed by D. Ritter (2/2/77). No ChE NOEL found. Valid study (HPB Canada). Classification - Core-Minimum Data.

5. Technical Chemical. Rabbit Oral LD_{50} - IBT#601-0481. LD_{50} = > 20, < 200 mg/kg. No validation report. (Artie Williams, SPRD tentatively has no record).

6. Formulation. Rat Oral LD_{50}. IBT#8380-10261. Invalid study (HPB Canada).

7. Formulation. Rat Dermal LD_{50}. IBT#8380-10261. Invalid study (HPB Canada).

8. Formulation. Rabbit Primary Eye Irritation. IBT#8350-10261. P.I. Index = 35.3/110.0. Toxicity Category I. Valid study (HPB Canada).. Classification - Core-Minimum Data.

9. Formulation. Rabbit Primary Skin Irritation. IBT#8350-10261. P.I. = 2.4 (moderate irritant). Toxicity Category - III. Valid study (HPB Canada). Classification - Core-Minimum Data.

10. Formulation. Rat Inhalation LC_{50}. IBT#8562-10260. LC_{50} = 11.5 mg/L air. Core Supplementary Data. Valid study (HPB Canada).

11. Formulation. Rabbit dermal LD_{50}. IBT#8350-10564. Invalid Study (HPB Canada).

12. Technical Chemical. Rat subacute oral. IBT#622-05122-B. Valid study (HPB Canada). Classification - Core-Minimum Study.

13. Technical Chemical. Dog 90-Day Feeding. IBT#8531-09996. No ChE NOEL found. HPB Canada validation in progress.

14. Formulation. Mouse Chronic Feeding. IBT#622-07923. No oncogenic potential. No ChE NOEL determined. Supplementary data for feeding study and Core-Minimum for oncogenic. (Validation by G. Burin 5/23/80).

15. Formulation. IBT No. 622-06895, Rat 2-Year Chronic Feeding. No oncogenic potential. Brain ChE NOEL = 0.08 ppm. LEL = 0.38 ppm. HPB Canada has requested additional information; Validation not complete.

From D. Ritter, TOX profile, 11/1/78. The acute/sensitization data listed below (Ritter TOX profile) is all IBT data (K. Locke, memo of June 14, 1979 - from report of R. Engler, 8/5/77; PP#7G1888) (these data have not been validated).

Acute/Sensitization Toxicity Data

16. Tech. Chemical - Rat LD_{50}, oral = 400 mg/kg BW, Tox. Cat. II,

17. Tech. Chemical - Rabbit LD_{50}, dermal = 472 mg/kg BW, Tox. Cat. II,

18. Tech. Chemical - Rat LC_{50}, inhalation = 2.6 mg/L, Tox. Cat. III,

19. Tech. Chemical - Rabbit Primary Skin Irritation (Draize) = 0.9/8, Tox. Cat. IV

20. Formulation (4 EC) - Rat LD_{50}, oral = 810 mg/kg BW, Tox. Cat. III,

21. Formulation (6E) - Rabbit LD_{50}, dermal = 241 mg/kg BW, Tox. Cat. II,

22. Use dilution - Rabbit LD_{50}, dermal 1:8 and 1:40 = 183 g/kg BW, Tox. Cat. III

23. Formulation (6E) - Rat LC_{50}, inhalation - > 2.45 mg/L, Tox. Cat. IV,

24. Formulation (6E) - Rat primary skin irritation (Draize) = 7.4/8, Tox. Cat. I

25. Formulation (6E) - Rat eye irritation (Draize) = 39/110, Tox. Cat. I,

26. Formulation (6E) - Guinea pig skin sensitivity = negative

Subacute Toxicity

27. Technical Chemical. 90-Day Dog Feeding Study (IBT#611-0592-A). No NOEL determined for RBC ChE at LDT of 2 ppm. Valid Study (HPB Canada). Core-Minimum Data

William S. Woodrow FDC 4/19/82

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Toxicology Branch
Hazard Evaluation Division (TS-769)

Diana Kaza DVM. Ph.D.

TS-769:th:TOX/HED:WSWoodrow:4-12-82:card 6

NOEL
Change not
recorded

	mg/kg		mg/kg/day	mg/day(60kg)
	[illegible]	[illegible]	10	0.0005	0.0300

Unpublished study received [illegible] 7210CB

CROP	Tolerance	Feed Factor	mg/day(1.5kg)
Cottonseed (Oil)(41)	[illegible]	0.15	[illegible]
Eggs(54)	[illegible]	2.77	[illegible]
Cattle, inc. poultry(02)	[illegible]	13.65	0.[illegible]
Milk&Dairy Products(93)	0.010	28.62	0.00429

TMRC	% ADI
[illegible] mg/day(60kg)	[illegible] mg/day(1.5kg)	7e.35

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Current Action [illegible]2057/[illegible]177

CROP	Tolerance	Feed Factor	mg/day(1.5kg)
Cottonseed (Oil)(41)	.050	0.15	0.00750
Eggs(54)	0.000	2.77	0.00000
Cattle, inc. poultry(02)	0.000	13.65	0.00000
Milk&Dairy Products(93)	0.000	28.62	0.00000

TMRC	% ADI
0.0300 mg/day(60kg)	0.0035 mg/day(1.5kg)	7e.35

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