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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

000821

DATE:

SUBJECT: EPA Reg.#100-EUP-AT (67), 9H5231, 9G2234 (CURACRON 6E INSECTICIDE - Soybeans), 100-L00, 100-L0I, 8F2057, 8H5177 (CURACRON - growing cotton). CASWELL#266AA. Acc. Nos. 093793, 097796, 097797, 097798, 097799, 098921, 097801, 097802, 097803, 097804, 097805, 097806, 097807.

FROM: William Woodrow, Ph.D
Toxicology Branch (TS-769)

TO: Ms. M. Mautz
Product Manager#16

THRU: M. Adrian Gross, Chief
Toxicology Branch (TS-769)

CC: Residue Chemistry Branch
(TS-769)

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

Residue Chemistry Branch Considerations:

1. PP#8F2057/FAP 8H5177. Curacron on cottonseed. Amendment of 2/14/79 from Donald Reed, RCB TS-769.

Recommendations by RCB

We defer to TOX regarding the adequacy of exposure, in the toxicity testing, to [redacted] of impurities which may be present in the technical material.

If TOX considerations permit, we recommend for establishment of tolerances for combined residues of the insecticide profenofos [O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl phosphorothionate] and its metabolites coverted to 4-bromo-2-clorophenol, and calculated as profenophos, in or on the following commodities:

R.A.C.'s

Cottonseed	3.0 ppm
Eggs and the meat, fat and meat by-products of cattle, goats, hogs, horses, poultry, and sheep	0.05 ppm
Milk	0.01 ppm

Processed feeds

Cottonseed hulls	6.0 ppm
Soapstock	15.0 ppm

2. PP#9G2234. Curacron on soybeans. From L. Bradley, RCB TS-769, 2/6/80:

Recommendations by RCB

"We recommend against estblishment of the proposed tolerances for the reasons stated in conclusions 2, 3b, 4b, and 5a (below). When the temporary tolerance is reviewed favorably by RCB, we will set forth our requirements for a permanent tolerance.

We have deferred to TOX in two previous petitions regarding the adequacy of exposure to the manufacturing impurities in the toxicity testing. We await their reply. Additionally, we defer ~~it~~ to TOX as to their concern with residue derived from the S-propyl moiety; propanethiol is the likely initial product of degradation."

Conclusions (RCB)

"2. The metabolism of profenofos in animals is not adequately understood for the purposes of these temporary tolerances. We will require further metabolic studies and metabolite identifications."

"3b. Due to the increased residue levels in animal feed items, we will require an analytical method which uses Tyrodes solution to extract profenofos metabolites from animal tissues."

"4b. The data for soybean fodder do not support the proposed tolerance of 55 ppm. Due to the wide variation in residue levels submitted, a tolerance level of 75 ppm would be more appropriate. Additionally, the tolerance proposal should be revised to read soybean straw as this is the more commonly accepted term for the commodity in question."

"5a. We are unable to make a conclusion concerning the adequacy of the proposed tolerance for residues in meat and milk at this time. Petitioner has previously been advised that tolerances on major livestock feed items would require higher level feeding studies than those submitted. We are now requesting those studies to support any tolerance on meat, milk and meat by-products, to be conducted using the analytical method which employs extraction with Tyrodes solution."

Recommendations by Toxicology Branch

1) The requested tolerance for use of Curacron on cotton and soybeans is not toxicologically supported.

2) A NOEL has not been established in the subchronic dog feeding studies. Another subchronic dog feeding study is required to provide the NOEL. It should be noted that this requirement is currently under revision by EPA and may be extended from 6-months to 2-years. In such case, this study (data gap) will have to comply with the amended requirement.

Tolerances Requested:

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

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Commodity	Proposed tolerances (ppm)

A. PP8F2057, PP8H5177 (100-L00, 100-L0I)

K.A.C.

Cottonseed	3.0
Eggs and meat by-products of cattle, goats, hogs, horses, poultry and sheep	0.05
Milk	0.01

Processed foods

Cottonseed hulls	6.0
Soapstock	15.0

B. PP9G2234, PP9H5231 (100-EUP-AT)

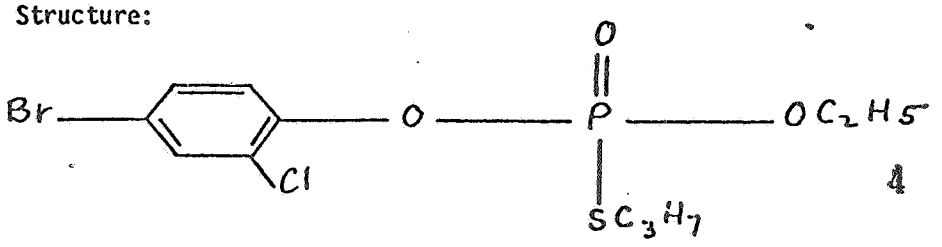
[redacted]

A. Substance Identification:

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

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

3. Structure:



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B. Formulation:

Curacron 6E Insecticide E.C.

Active Ingredient (Confidential)	Percent Weight
Profenofos (technical 88%)	67.8%
Inerts (Confidential)	[redacted]

Inerts cleared under 180.1001(c).

C. Previously Submitted Toxicity Data. (From D. Ritter, TOX profile, 11/1/78.)

Acute/Sensitization Toxicity Data

Tech. Chemical - Rat LD50, oral = 400 mg/kg BW, Tox. Cat. II, Core-Minimum Data

Tech. Chemical - Rabbit LD50, dermal = 472 mg/kg BW, Tox. Cat. II, Core-Minimum Data

Tech. Chemical - Rat LC50, inhalation = 2.6 mg/L, Tox. Cat. III, Core-Minimum Data

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

Formulation (4 EC) Rat LD50, oral = 810 mg/kg BW, Tox. Cat. III, Core-Minimum Data

Formulation (6E) Rabbit LD50, dermal = 241 mg/kg BW, Tox. Cat. II, Core-Minimum Data

Use dilution - Rabbit LD50, dermal 1:8 and 1:40 = 183 g/kg BW, Tox. Cat. III, Core-Minimum Data

Formulation (6E) Rat LC50, inhalation - > 2.45 mg/L, Tox. Cat. IV, Core-Minimum Data

Formulation (6E) Rabbit primary skin irritation (Draize) = 7.4/8, Tox. Cat. I, Core-Minimum Data

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

Formulation (6E) Guinea pig skin sensitivity = negative Core-Minimum Data

Subacute Toxicity

1. 90-Day Rat Feeding Study (Hazleton #1519).

   No NOEL (3 ppm lowest test) determined for RBC or brain ChE activity.

   Core-Minimum Data

2. 90-Day Dog Feeding Study (IBT#611-05122-B). TECH

   No NOEL determined for RBC ChE at lowest dose of 2 ppm.

   Core-Minimum Data

Special Neurotoxicity Study (IBT#8580-10426).

Birds treated with 38% a.i. formulation. 2-21 day successive treatments - doses to 44.5 mg/kg a.i. No neurotoxic signs, or histopathological evidence of delayed neurotoxicity.

Core-Minimum Data

Chronic Rat Feeding, 1-Year Interim Report.

Report stated no adverse clinical effects at up to 200 ppm Curacron; did show ChE effects at 20 ppm, but not at 1 or 0.2 ppm. (Later determined that 38% E.C. used, instead of intended tech. chemical; above values are in error).

Previously Submitted Toxicity Data (Memo from Mary Quaife, 6/13/79).

Dr. Quaife made a cursory check of the 2-year rat feeding, 18-month mouse oncogenicity, and multi-generation rat reproduction studies; IBT acknowledged that a 38% E. concentrate was used in the 2-year rat feeding and 18-month mouse oncogenic studies - tech. chemical used in multi-generation rat reproduction studies.

Results (Dr. Quaife)

1. Reproduction and oncogenicity studies appeared unremarkable.

2. The 2-yr. rat feeding study appeared to show brain ChE inhibition in rats at "1" ppm and RBC and plasma ChE inhibition in rats at "20 ppm". Accounting for the 38% a.i. used, corresponding NOEL's would actually be 0.076 ppm - brain ChE; and 0.38 ppm - RBC and plasma ChE.

   This finding (ChE) for rat 2-year study at 0.076 ppm (lowest dose tested) was concurred by Ciba-Geigy in letter from J. Harrison to C. Smith, EPA, 7/20/77.

D. New Curacron Toxicity Studies (See review section this report)

Acute Toxicity

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

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

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

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Mouse Oral LD_{50} = 298 mg/kg. Toxicity Category II, Core-Guidelines 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 - IBT No. 601-0481, Acc. No. 097794. Rabbit Oral LD_{50} = > 20, < 200 mg/kg. Supplementary 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, TOX Cat. III

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Rabbit Primary Eye Irritation, P.I. Index = 0.4. Supplementary Data, TOX Cat. III

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

Formulation (CGA-15324 6E) - I.B.T. No. 8350-10261, Acc. No. 097793. Rat Oral LD_{50} = 662.03 mg/kg. Toxicity Category III, Core-Minimum Data

Formulation (CGA-15324 6E) - I.B.T. No. 8350-10261, Acc. No. 097793. Rabbit Dermal LD_{50} = 192.37 mg/kg. Supplementary Data, TOX Cat. I

Formulation (CGA-15324 6E) - I.B.T. No. 8350-10261, Acc. No. 097793. Rabbit Primary Eye Irritation, P.I. Index = 35.3/110.0. Toxicity Category I, Core-Minimum Data

Formulation (CGA-15324 6E) - I.B.T. No. 8350-10261, Acc. No. 097793. Rabbit Primary Skin Irritation, P.I. Index = 2.4 (moderate irritant). Toxicity Category III, Core-Guidelines Data

Formulation (CGA-15324 6E) - I.B.T. No. 8350-10564, Acc. No. 097793. Rabbit Dermal LD_{50} = 236.17 mg/kg. Toxicity Category II, Core-Minimum Data

Formulation (CGA-15324 6E) - I.B.T. No. 8562-10260, Acc. No. 097793. Rat Inhalation LC_{50} = 11.5 mg/L air. Supplementary Data

Subacute, 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. Toxicity Category IV, Core-Minimum 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. 09779. Chicken Delayed Neurotoxicity. Acute Chicken Oral LD_{50} = 35 mg/kg. No delayed neurotoxicity. Supplementary Data

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Tech. Chemical - I.B.T. No. 8580-11187, Acc. No. 097797. Chicken Delayed Neurotoxicity. Acute Chicken Oral LD_{50} = 45.7 mg/kg. No delayed neurotoxicity. Core-Minimum 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 - Ciba-Geigy No. 22741900, Acc. No. 097797. Rat Teratogenic Evaluation. Not teratogenic. Core-Guidelines 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

Tech. Chemical - I.B.T. No. 611-05122-B, Acc. No. 097796. Dog Subacute Oral Feeding "Final Report" - previously reviewed by D. Ritter, 2/2/77. No ChE. NOEL found. Core-Minimum Data

Tech. Chemical - I.B.T. No. 8531-09996, Acc. No. 097796. Dog 90-Day Feeding. No ChE. NOEL found. Supplementary Data

Tech. Chemical - I.B.T. No. 623-07924, Acc. No. 097798. Three-Generation Reproduction, Rat. No compound-related reproductive effects (highest dose 20 ppm). RBC ChE. NOEL = 1.0 ppm. Core-Minimum Data

Formulation (CGA-15324 38% E.C.) - I.B.T. No. 8580-10426, Acc. No. 097797. Chicken Delayed Neurotoxicity. No delayed neurotoxicity found. No ChE. NOEL determined. Core-Minimum Data

Chronic Toxicity

Tech. Chemical - Hazelton Labs., Acc. No. 09821. One-Year Progress Reports: #483-133 - Mice, #483-134, Rat; Chronic Feeding Studies. Mice - not carcinogenic, tentative RBC ChE. = 0.38 ppm. Rats - no compound-related effects. RBC ChE. inhibition at lowest dose - 0.38 ppm. No classified at this time.

Formulation (CGA-15324 38% E.C.) - I.B.T. No. 622-07923, Acc. No. 097799. Mouse Chronic Feeding. No oncogenic potential. No ChE. NOEL determined. Supplementary Data for feeding study and Core Minimum for oncogenic.

Formulation (CGA-15324 38% E.C.) - I.B.T. No. 622-06895, Acc. No. 097801, 097802, 097803, 097804, 097805, 097806, 097807. Rat 2-Year Chronic Feeding. No oncogenic potential. Brain ChE. NOEL = 0.08 ppm. LEL = 0.38 ppm. Core-Minimum Data

E. Review of New Toxicity Studies

Acute Toxicity

Tech. Chemical - Ciba-Geigy No. 6021, Acc. No. 097794. Rat Oral LD_{50}. May 12, 1977, Batch No. EN 31664.

Animals were dosed with Technical CGA-15324 diluted with 2% carboxymethyl cellulose. 5M and 5F animals/dose group were treated by intubation with 100, 215, 359, or 600 mg/kg. Animals observed 14 days.

Dose mg/kg	Died Within 1 Hour		24 Hours		48 Hours		7 Days		14 Days	
100	0	0	0	0	0	0	0	0	0	0
215	0	0	0	0	1	0	1	0	1	0
359	0	0	0	0	0	1	2	3	2	3
600	0	0	0	0	3	3	5	5	5	5

Surviving animals recovered within 8 to 13 days.

Acute Oral LD_{50}, rats (both sexes) = 334 (277 - 401) mg/kg (65% C.L.)

Toxicity Category: II

Classification: Core-Guidelines Data

Tech. Chemical - Ciba-Geigy No. 6020, Acc. No. 097794. Rat Oral LD_{50}. June 1, 1977, Batch No. EN 31665.

Technical CGA-15324 was diluted with 2% carboxymethyl cellulose, administered by intubation. Groups of 5M and 5F rats/dose level were treated with 167, 215, 278, 359, 600, or 1000 mg/kg. Animals observed 14 days.

Results

Dose mg/kg	Died Within 1 Hour		24 Hours		48 Hours		7 Days		14 Days	
167	0	0	0	0	0	0	0	0	0	0
215	0	0	0	0	0	0	0	0	0	0
278	0	0	0	0	3	1	3	1	3	1
359	0	0	0	0	2	4	3	4	3	4
600	0	0	0	0	2	2	5	3	5	3
1000	0	0	2	2	3	3	5	5	5	5

Acute Oral LD_{50}, rats = 344 (284 - 416) mg/kg. (95% C.L.)

Toxicity Category: II

Classification: Core-Guidelines Data

Tech. Chemical - Ciba-Geigy No. 6019, Acc. No. 097794. Rat Oral LD_{50}. June 1, 1977, Batch No. 31.

Technical CGA-15324 was diluted with 2.0% carboxymethyl cellulose and administered by intubation. Groups of 5M and 5F rats/dose level were treated with 167, 278, 359, 600, 775, or 1000 mg/kg, and observed 14 days.

Results — Died Within

Dose mg/kg	1 Hour		24 Hours		48 Hours		7 Days		14 Days	
167	0	0	0	0	0	0	0	0	0	0
278	0	0	0	0	0	0	0	1	0	1
359	0	0	0	0	2	1	3	2	3	2
600	0	0	0	0	2	2	3	2	3	2
775	0	0	0	0	2	4	5	5	5	5
1000	0	0	2	1	4	5	5	5	5	5

Acute Oral LD_{50} = 447 (366 - 548) mg/kg (rats) (95% C.L.)

Toxicity Category: II

Classification: Core-Guidelines Data

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Mouse Oral LD_{50}. Jan. 22, 1974, Batch No. mg 1.

CGA-15324 technical was diluted with 2% carboxymethyl cellulose and administered by intubation.

Groups of 5M and 5F mice were separately dosed with 215, 278, 317, or 464 mg/kg, and observed 14 days.

Results — Died Within

Dose mg/kg	2 Hours		24 Hours		48 Hours		7 Days		14 Days	
215	0	0	1	0	1	0	1	0	1	0
278	0	0	1	0	2	1	2	1	2	1
317	1	0	1	0	5	1	5	1	5	1
464	0	1	4	3	5	5	5	5	5	5

Acute Oral LD_{50}, mice, CGA Technical = 298 (268 - 332) mg/kg (both sexes) 95% C.L.

Toxicity Category: II

Classification: Core-Guidelines Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Mouse Oral LD_{50}. August 24, 1973.

Technical CGA-15324 was diluted with 2% carboxymethyl cellulose and administered via intubation to mice.

Groups of 5M and 5F mice were separately treated with 317, 359, or 464 mg/kg, animals observed 7 days.

Results — Died Within

Dose mg/kg	1 Hour		24 Hours		48 Hours		7 Days	
317	0	0	1	0	1	0	1	0
359	0	0	1	0	3	2	4	5
464	0	1	2	4		4	5	4

Acute Oral LD_{50}, mice, CGA-15324 Technical = 336 (325 - 348) mg/kg C.L.= 95%.

Toxicity Category: II

Classification: Core-Guidelines Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rabbit Oral LD_{50}. June 4, 1973.

Technical CGA-15324 was diluted in polyethylene glycol (20%) and administered to rabbits by intubation.

2M and 2F rabbits/group were separately dosed with 215, 359, 464, or 600 mg/kg. Animals observed 7 days.

Results — Died Within

Dose mg/kg	1 Hour		24 Hours		48 Hours		7 Days	
215	0	0	0	0	0	0	0	0
359	0	0	0	1	1	1	2	1
464	0	0	1	1	1	2	2	2
600	0	0	2	2	2	2	2	2

Acute Oral LD_{50}, rabbits = approximately 300 mg/kg.

Toxicity Category: II

Classification: Core-Minimum Data (Animals should have been observed 14 days).

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Rabbit Oral LD_{50}. April 1, 1974, Batch No. mg 1.

Technical CGA-15324 was diluted with 2% carboxymethyl cellulose administered by intubation.

Groups of 2M and 2F rabbits were separately treated with 100, 600, 1000, or 2150 mg/kg and observed 7 days.

Results

Dose mg/kg	Died Within 1 Hour		24 Hours		48 Hours		7 Days	
100	0	0	0	0	0	0	0	0
600	0	0	0	0	1	0	1	0
1000	0	0	1	2	2	2	2	2
2150	0	0	2	2	2	2	2	2

Acute Oral LD_{50}, rabbits = approximately 700 mg/kg.

Toxicity Category: III

Classification: Core-Minimum Data (Observation should have been 14 days).

Tech. Chemical - I.B.T. No. 601-0481, Acc. No. 097794. Rabbit Acute Dermal LD_{50}. April 3, 1974, Batch No. FL 740205.

Groups of 4M and 4F rabbits were treated on intact and abraded skin sites under occlusive dressing for 24 hours. Skin sites washed after 24 hours, animals observed for 14 days; excepting those animals in the lowest test group which were placed on test several days following the higher dose groups.

2M and 2F rabbits/test group treated at intact skin sites, 2M and 2F rabbits treated at abraded skin sites.

Results

Dose mg/kg	Number Dead/ Number Tested	Number Dead
20	0/8	0
200	8/8	100
1000	8/8	100

CGA-15324 technical was mildly irritating at a level of 20 mg/kg, with well defined erythema and mild edema at 24 hours, mild to moderate desquammation at 7 days, and slight desquammation at 14 days. (Intact and abraded animals.)

Acute Dermal LD_{50} of CGA-15324 technical, rabbits, with intact or abraded skin in > 20, < 200 mg/kg.

Toxicity Category: I

Classification: Supplementary Data (Not enough dose levels to determine an LD_{50}; 20 mg/kg animals placed on test several days following test beginning with remaining animals).

Tech. Chemical - Ciba-Geigy No. 5048, Acc. No. 097794. Rat I.P. LD_{50}. December 2, 1975, Batch No. P-8, A-D.

Technical CGA-15324 was diluted with 2% carboxymethyl cellulose; doses of 100, 215, 464, 600, 775, 1290 mg/kg were administered I.P. separately to groups of 5M and 5F each. Animals observed 14 days.

Results — Died Within

Dose mg/kg	2 Hours		24 Hours		48 Hours		7 Days		14 Days	
100	0	0	0	0	0	0	0	0	0	0
215	0	0	0	0	0	0	0	0	0	0
464	0	0	0	0	0	0	0	1	0	1
600	0	0	0	0	5	1	5	1	5	1
775	0	0	1	0	3	4	3	5	3	5
1290	0	0	4	4	5	5	5	5	5	5

Acute intraperitoneal LD_{50} of CGA-15324 in rats (both sexes) = 585 (500 - 685) mg/kg. 95% C.L.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rat Dermal LD_{50}. June 26, 1973.

Technical CGA-15324 was tested on intact skin, shaved backs of 9M and 9F rats/dose level of 1 000, 1470 and 3170 mg/kg each.

Treated sites were maintained under occlusive dressings for 24 hours, then washed. Animals were observed for 7 days.

Results — Died Within

Dose mg/kg	1 Hour		24 Hours		48 Hours		7 Days	
1000	0	0	0	0	0	1	1	2
1470	0	0	0	0	0	1	1	2
3170	0	0	1	1	1	2	2	2

Acute Dermal LD_{50} technical CGA-15324, rats = 1610 (1073 - 2415) mg/kg. 95% C.L.

Toxicity Category: II

Classification: Core-Minimum Data (Animals should have been observed for 14 days, toxic symptoms should have been described).

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rat Inhalation LC_{50}. July 12, 1973.

Technical CGA-15324 was sprayed into an exposure chamber at rates of 6, 30, 60 ml/hr., or water only for a control; to create test chamber concentrations of 736, 2052, or 2147 mg/m^3. Groups of 9M and 9F rats were separately exposed to the above concentrations for 1 hour, and were then observed for 7 days post exposure. The spray particles size was determined gravimetrically. Air flow 7.5 L/minute, particle distribution = < 1 - 77 u.

Results

conc., mg/m^3	Died Within 0 - 1 Hour		24 Hours		48 Hours		7 Days	
736 ± 40	0	0	0	0	0	0	0	0
2052 ± 40	0	0	0	0	0	0	0	0
2147 ± 32	0	0	0	0	0	0	0	0
water	0	0	0	0	0	0	0	0

After 1 hour exposure animals at highest concentration showed trachypnoea, exothalmus, trismus, apathy, and ruffled fur. All symptoms disappeared within 24 hours.

Acute Inhalation LC_{50}, technical CGA-15324, rats = > 2150 mg/m^3, or > 2.150 mg/L air.

Toxicity Category: III

Classification: Supplementary Data (Animals should have been observed at least 14 days, and either a higher dose concentration or longer exposure period(s) should have been tested).

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Rat Inhalation LC_{50}. June 2, 1974, Batch No. mg 2.

Separate groups of 9M and 9F rats were exposed to technical CGA-15324 at dose levels of 643, 1457, or 2624 mg/m^3 in an exposure chamber. Each group exposed for 4 hours. Animals observed 7 days post exposure. No water control group of rats was included.

Test liquid was pressure injected at rats of 12, 30, and 60 ml/hour into a stream of compressed air flowing through a spray nozzel at a rate of 10 l/minute. Particle size distribution was determined.

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Results — Died Within

conc, mg/m^3	0 - 4 Hours		24 Hours		48 Hours		7 Days	
643 ± 22	0	0	0	0	0	0	0	0
1457 ± 29	0	0	1	0	1	0	1	0
2624 ± 220	0	0	2	2	2	2	2	2

Particle size distribution ranged from < 1 to > 7 u.

Symptoms - following 4 hours exposure, rats exposed to two highest concentrations showed dyspnoca, exthalmus, slight tremor and ruffed fur. Survivors recovered within 3 days.

Acute Inhalation LC_{50} for CGA-15324 technical, rat (both sexes exposed 4 hours = 3000 mg/m^3 of air, which is = to 3.00 mg/L of air.

Toxicity Category: III

Classification: Core-Minimum Data (Animals were exposed for 7 days only, and a water exposed control group should have been included).

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rabbit Primary Eye Irritation. June 25, 1973.

0.1 ml undiluted test material instilled into conjunctival sac of left eye of rabbits. Right eyes served as controls. Treated eyes of 3 rabbits rinsed with water 30 seconds after treatment. Reactions appraised after 24 hours, 2, 3, 4 and 7 days post treatment according to Draize.

Results

Only one rabbit eye reacted at conjunctivae (unwashed eye). Primary irritation index = 0.2. Compound tentatively found slightly irritating.

Toxicity Category: III

Classification: Supplementary Data (Should have used 9 animals on test).

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Rabbit Primary Eye Irritation. March 11, 1974. Batch No. mg 1.

0.1 ml of technical CGA-15324 was introduced into the conjunctival sac of 3M and 3F rabbits. Right eyes served as controls. Treated eyes of 3 rabbits were each washed with water 30 seconds after treatment. Reactions were appraised after 24 hours, 2, 3, 4 and 7 days post treatment according to Draize.

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Results

No reactions or other symptoms in rabbits with rinsed eyes. Two of 3 rabbits with unwashed eyes died on days 2 and 3 post treatment. The conjunctival sac of one these rabbits, was affected on day 1. A primary irritation score of 0.4 was calculated for this one irritation response.

Toxicity Category: III

Classification: Supplementary Data (No explanation given for two mortalities - not enough animals used in test).

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Rabbit Primary Skin Irritation. June 25, 1973.

0.5 ml CGA-15324 technical chemical undiluted applied in guaze patches to abraded and intact skin sites on each of 3M and 3F rabbits.

Patches under occlusive dressings for 24 hours, after which they were removed. Skin reactions were appraised 24 and 72 hours post treatment and scored according to Draize.

Results

Primary skin irritation index of CGA-15324 technical was 0; however, 5 of 6 treated animals died within 3 days post treatment. No recovery of severe lateral and curved position, asynchronism of the extremities, slight muscular spasms and apathy in the surviving rabbit.

Classification

Supplementary Data (It would appear that the compound does not cause significant skin irritation, but is able to penetrate the epidermis and produce what appear to be neurotoxic disorders.)

Formulation (CGA-15324 6E E.C.) - I.B.T. No. 8350-10261, Acc. No. 097793. Rat Oral LD_{50}. Feb. 16, 1977, Batch No. FL-770021; ARS No. 9/77.

Test Material - CGA-15324 6E E.C.

Test material administered as 10.0% w/v solution at 266.7 and 400 mg/kg dose levels; undiluted material used in remaining doses.

5 groups of 2M and 2F rats each were separately dosed by intubation with 266.7, 400.0, 600.0, 900.0, or 1350 mg/kg of CGA-15324 6E formulation. Animals observed 14 days.

Results

Dose	Dead/Total	% Dead
266.7	0/4	0
400.0	0/4	0
600.0	2/4	50
900.0	3/4	75
1350.0		100

Acute Oral LD_{50}, Rats = 662.03 mg/kg. 95% C.L. = 459.6 - 953.6 mg/kg.

Toxicity Category: III

Classification: Core-Minimum Data

More animals/test group/sex should have been used. A control group treated with vehicle alone should have been included. No distribution in sex of dying (dead) animals was made.

Formulation (CGA-15324 6 EC) - I.B.T. No. 8350-10261, Acc. No. 097793. Rabbit Dermal LD_{50}. Feb. 16, 1977, Batch No. FL-770021, ARS No. 9/77.

Test Material: CGA-15324 6E, undiluted.

6 groups of 2M and 2F rabbits each were separately treated by the dermal route with the following dose levels: 79.0, 118.5, 177.8, 266.7, 400, or 600 mg/kg. Method of dosage application and length of contact time were not stated. Animals were observed 14 days post-treatment.

Results

Dose Level mg/kg	Dead/Total	% Dead
79.0	0/4	0
118.5	2/4	50
177.8	2/4	50
266.7	2/4	50
400.0	3/4	75
600.0	4/4	100

Acute Dermal LD_{50}, Rabbits = 192.37 mg/kg. 95% C.L. = 104.2 - 355.0 mg/kg.

Toxicity Category: I

Classification: Supplementary Data

Additional animals should have been used/sex/dosage level. A vehicle control should have been included. A complete description of the amount (volume) applied means of securing treatment during contact period, and the length of the contact period should have been stated.

Formulation (CGA-15324 6 EC) - I.B.T. No. 8350-10261, Acc. No. 097793. Rabbit Eye Irritation. Feb. 16, 1977, Batch No. FL-770021, ARS No. 9/77.

Test Material: CGA-15324 6E formulation.

0.1 ml of undiluted test material was instilled into 1 eye each of 6 rabbits; untreated eyes served as controls. Treated eyes of 3 rabbits were washed after 30 second exposure. Eyes were scored according to Draize for toxic effects at 1, 24, 48 and 72 hours, and after 7 and 14 days post-treatment.

Results

Unwashed Eyes - Maximum mean irritation score = 35.3/110.0. Corneal opacity and conjunctival injury persisted through 7 days.

Washed Eyes - Maximum m ean irritation score = 20.3/110. All toxic effects disappeared at 72 hours.

Toxicity Category: I

Classification: Core-Minimum Data

Should have used 9 animals.

Formulation (CGA-15324 6 E.C.) - I.B.T. No. 8350-10261, Acc. No. 097793. Rabbit Primary Skin Irritation. Feb. 16, 1977, Batch No. FL-770021, ARS No. 9/77.

Test Material: CGA-15324 6E formulation.

0.5 ml undiluted test material applied to intact and abraded skin sites on 6 rabbits. Test material loss was minimized by gauze pads under occlusive dressing for 24 hours contact. Treated sites were scored according to Draize at 24 and 72 hours.

Results

Primary Irritation Score = 2.4 (a moderately irritating material).

Toxicity Category: III

Classification: Core-Guidelines Data

Formulation (CGA-15324 6 EC) - I.B.T. No. 8350-10564, Acc. No. 097793. Rabbit Dermal LD_{50}. April 8, 1977, Batch No. FL-770021, ARS No. 9/77.

Test Material: CGA-15324 6E formulation.

Undiluted test material (volumes not stated) was applied to abraded and intact skin sites on rabbits. Groups of 5M and 5F rabbits were separately treated with 118.5, 177.8, 266.7, or 400 mg/kg of test material. The skin of 3M and 2F/group was abraded prior to testing. Test applications were occluded with plastic sleeves for 24 hours contact, followed by washing. Animals observed for 14 days.

Results

Dose	Dead/Tested	% Dead
118.5	1/10	10
177.8	1/10	10
266.7	7/10	70
400.0	9/10	90

Acute Dermal Toxicity, Rabbits, LD_{50} = 236.17 mg/kg, C.L. (95%) = 181.2 - 207.8 mg/kg.

Toxicity Category: II

Classification: Core-Minimum Data

Formulation (CGA-15324 6 EC) - I.B.T. No. 8562-10260, Acc. No. 097793. Rat Inhalation LC_{50}. Feb. 14, 1977

Test Material: CGA-15324 6 EC

Aerosol generated by nebulization of undiluted test material combined with clear, dry air (-40°C dewpoint) which was introduced into an 80 liter inhalation chamber through the top. Each animal (5M and 5F rats) was separately housed in the exposure chamber, and was exposed whole body for a period of 4 hours to a single dose of 11,500 mg/m^3.

The test dose was determined by repeatedly passing the aerosol through glass sampling filters and dividing the total weight of trapped test material by the air drawn through filters. Atmospheric pressure was 29.38, temperature 26°C, air flow/minute = 8.57 liters.

Results

All animals survived. Hypoactivity noted in all animals 5 minutes after beginning test; This reaction disappeared within 18 hours. Salivation noted 40 minutes into exposure, which subsided by day 3 of 14 day observation period. No other reactions. Actual concentration/liter:

$$\frac{11{,}500\ mg/m^3}{1000\ liters} = 11.5\ mg/liter =$$

concentration in exposure chamber.

Toxicity Category: III

Classification: Supplementary Data

An LC_{50} was not determined, at least 4 dosage levels should have been used, and a concurrent control group exposed to vehicle alone should have been included.

Subacute, Subchronic and other Non-Chronic Toxicity Studies

Tech. Chemical - Ciba-Geigy No. 5119, Acc. No. 097797. Rabbit 21-Day Dermal Tox. October 19, 1976.

Test Material - CGA-15324 technical chemical. Batch No. 11. 89.8% pure. CGA-15324 diluted daily in polyethylene glycol (PEG 400) and saline (70:30 parts).

Groups of 3M and 3F rabbits treated daily by the dermal route with 0, 5, 20, or 100 mg/kg of test solution in gauze patches under occlusive dressings 5x/week for 3 weeks. Treatment contact/day not stated. One male and 1 female/group were observed 21-days after treatment stopped. Irritation scores determined according to Draize.

Hematologic, blood chemistry and urinalyses studies conducted.

Macroscopic examinations were conducted at termination of the experiment, after recovery period, or following spontaneous deaths. After exsanguination, rabbits weighed, selected organ/body wt. and organ/brain weight ratios calculated.

Histopathologic examination of selected tissues and organs including spinal cord and eye ball with optic nerve conducted.

AChE activity in plasma and RBC and measured at day 4, 10, 26 and 33. At 21-days and at termination, brain ChE activity determined.

Results

Food Consumption - 100 mg/kg treatment group - food consumption dropped continuously until death at end of 1st week. Food intake of other groups comparable to that of controls. Body wt. of treated and control groups dropped during 1st week of treatment. All groups (except 100 mg/kg) made comparable gains thereafter to end of experiment and recovery period.

Hematology - No treatment related changes were observed.

Blood Chemistry - Plasma ChE levels very significantly depressed at all test levels (5, 20, and 100 mg/kg). RBC ChE levels significantly depressed at 100 mg/kg by day 4, depressed at all levels (5, 20, and 100 mg/kg) for remainder of experiment. Depressed ChE activity returned to that of untreated controls in surviving animals by end of 21-days.

NOTE: A NOEL for ChE inhibition was not determined in this experiment.

Organ Weights - No obvious differences in absolute or relative organ weights.

Macroscopic Pathology

Five of 6 rabbits treated with 100 mg/kg/day died spontaneously during the first test week (4th to 6th day). In all of these animals local edema and swelling of the skin and subcutis at the site of application and acute congestion of the liver with yellowish patches was observed. No compound related gross pathology was noted in the treated rabbits from the 5 and 20 mg/kg/day dosage groups.

Histopathological Findings

Acute congestion of the liver, focal hypertrophy of hepatocytes, dispersed areas of recent necropsies of liver parenchyma with hemorragha - and fatty changes of adjacent hepatocytes were detected in the 100 mg/kg test group. Slight or moderate atrophy of the lymphoid and thymic tissue was observed in these rabbits as well. No compound related histological findings observed in rabbits from 5 and 20 mg/kg groups.

Conclusions:

1) 100 mg/kg of test material resulted in death of 5 of 6 rabbits by the dermal route, accompanied by gross and microscopic evidence of severe liver damage, erythema and edema at application sites.

2) No NOEL for cholinesterase inhibition was determined (lowest dose tested was 5 mg/kg by dermal route).

Classification: Supplementary Data

Tech. Chemical - Ciba-Geigy No. 5119, Acc. No. 097797. Rat 21-Day Inhalation. Jan. 5, 1977, Batch No. 11.

Test Material: Groups of 9M and 9F rats were separately exposed to 0, 68, 219, or 449 mg/m^3 of the technical chemical 6 hrs/day, 5 days/week for 3 weeks.

NOTE: In terms of mg/liter of aerosol, the above values become 0, 0.068, 0.219, or 0.449.

4M and 4F rats/group were observed 21-days post treatment, during a recovery period.

Aerosols generated by injecting test material at a rate of 0.19, 0.6, and 1.2 ml/hr. into an air stream discharged into an exposure chamber through a spray nozzel under 2 atmosphere pressure.

Control rats treated with water aerosol. Particle sizes were determined.

Complete clinical observations made, opthalmic examinations performed weekly. When necessary, differences between treated groups and controls determined by student's t test.

Hematologic, urinalysis and blood chemistry measured at end of 21-day treatment, and on selected rats at end of recovery period. Gross and microscopic pathology studies were conducted.

Results

Mortality:

Dose mg/L	% Dead
0	0*
0.068	0
0.219	5.5
0.449	100.0

*1 animal died during blood collection.

All rats of group 4 (0.449 mg) and one female rat of group 3 (0.219 mg) died during 1st week of experiment. Food intake of male rats of group 3 decreased during entire exposure period, whereas wts. of females of this group and all rats in group 1 (0.068 mg) decreased during 1st exposure week only. Animals of group 4 lost wt. until they died. Food intake and body wt. gain of males of group 3 (depressed during treatment period) was comparable to controls by end of recovery period.

Hematological and blood chemistry analyses were generally unremarkable for both treated and control rats.

The cholinesterase activity of plasma, RBC, and brain was significantly depressed in all groups treated with CGA-15324 in a dose related fashion:

Dose mg/L	% cholinesterase activity at test day-21					
	ChE		AChE		Brain	
	Male	Female	Male	Female	Male	Female
0	100	100	100	100	100	100
0.068	65	53	36	47	39	50
0.219	58	42	29	36	20	35
0.449	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

NOTE: A cholinesterase NOEL was not determined in this subchronic 21-day inhalation study.

The total plasma protein level of all treatment groups was slightly lower than controls when analyzed statistically.

Cholinesterase values for test animals were comparable to the controls at the end of the recovery period; however, in the case of animals at the 0.219 mg/L level, about 25% inhibition was still demonstrated for plasma and brain cholinesterase at the end of the 21-day recovery period.

No differences in absolute and relative organ weights between treated and control rats, except for increased organ to body wt. ratios of the male rats of groups 2 and 3.

Acute congestion of nasal mucous membrance and some intermittant or purulent keratitis in all rats at the highest test concentration in animals that died on 3rd to 5th test day.

Pathology

Macroscopic - Autopsy of 0.449 mg/L rats upon death showed acute congestion of all groups. Animals of the 0.219 mg/L group were slightly emaciated. Due to reduced body wts. of groups 2 and 3 animals at termination of the study the organ to body wt. ratios were increased in these animals, whereas the organ to brain wts. were comparable to controls.

Microscopic - In rats receiving 0.449 mg/L (which died), in addition to acute congestion of the parenchymatous organs, marked congestions of the nasal mucous membrane, acute conjunctivitis and in most animals severe interstitial or purulent keratitis was seen. Rats of all the treated groups showed only incidental findings.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy Test, Acc. No. 097797. Feb. 15, 1977. Curacron (CGA-15324) tested with methidathion or diazinon for potentiation.

A. Acute Oral LD_{50}'s experimentally determined in rats for each compound.

B. Acute Oral LD_{50} values for equitoxic mixtures of CGA-15324 and each of the other 2 insecticides was determined and compared with theoretical additive LD_{50} values.

A. 5M and 5F rats per dose were separately administered 14.7, 21.5, 31.7, 46.4, 60.0, or 77.5 mg/kg of CGA-13005 (methidathion). Animals observed 14 days.

5M and 5F rats per dose group were separately administered 464, 600, 775, 1000, 1470, or 1670 mg/kg of G24480 (diazinon). Animals observed 14 days.

B. Equitoxic mixtures - of CGA-15324 and GS-13005; or CGA-15324 and GS-13005.

5M and 5F/dose level separately administered 77.5, 100.0, 128, 167, 215, or 278 mg/kg of CGA-15324 and G24480. Animals observed 14 days post treatment with equitoxic mixtures. 5M and 5F/dose level were separately administered 278,359, 464, 600, 775, 1000, or 1290 mg/kg of CGA-15324 and GS-13005. Animals observed 14 days post treatment.

Theoretical combined equitoxic mixture LD_{50} values were compared with actual tests of each compound individually performed.

Results

A. Individual LD_{50} values:

1. CGA-15324 Acute Oral LD_{50} = 279 (244-319) mg/kg.

2. CGA-13005 Acute Rat Oral LD_{50} = 38 (29-49) mg/kg.

3. G24480 Acute Rat Oral LD_{50} = 806 (201-927) mg/kg.

B. Combined CGA-15324 + GS-13005:

1. Acute Rat Oral LD_{50} = 128 (113-145) mg/kg

2. Combined CGA-15324 + GS24480 Acute Rat Oral LD_{50} = 602 (515-704) mg/kg.

Comparison:

	Theor. LD_{50} mg/kg	Exp. LD_{50} mg/kg	ratio theor./exptl.
CGA-15324+	158.5	128 (113-145)	1.23
GS-13005	542.5	602 (515-704)	0.90

Conclusion

Acute oral toxicity of CGA-15324 was not potentiated when administered in combination with either of the 2 insecticides.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy Test, Acc. No. 097797. CGA-15324; Antagonism of Atropine or Toxogonin.

a. Determination of LD_{50} of technical CGA-12223 (project No. Siss 3651), and CGA-15324 (Project No. 3647) Ciba-Geigy, Basle, Switerland.

b. Determination of the antagonistic effects of atropine or toxogonin administered singly or in combination by various routes, prior to or after administration of insecticides; with respect to effects of the two insecticides administered orally at an LD_{50} level. 5M and 5F rats/dose level were used. Animals were observed for 14 days, records maintained daily for toxicity signs and mortality.

Results

Within 2 hours rats in all dosage groups in all tests showed sedation, dyspnea, exothalmus, curved position, toxic-clonic muscle spasms and ruffled fur. With respect to CGA-15324, female rats were more susceptible than males.

At autopsy, no substance related gross organ changes were seen.

In rats, toxigonin was shown to effectively antagonize the toxic actions of CGA-12223. Atropine and toxigonin administered together produced a slight protective response: particularly, in case of intraperitoneal application the treatment proved effective. These observations indicate that toxigonin alone produced the most effective therapeutic response against effects of CGA-12223. Atropine alone was not effective.

The toxic effects of CGA-15324 were neither antagonized by atropine or toxigonin alone or in combination.

Classification: Core-Minimum Data

The therapeutic activity of pralidoxin (pam) and toxigonin regarding CGA-15324 in the rat were investigated.

Single, 3x or 5 repeated challenges of CGA-15324 LD_{50} doses were employed to test protective effects of pam, toxogonin, or combinations of pam and toxogonin adminsitered by the IM route at different dose levels after the appearance of poisoning signs.

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Results

When toxogonin was administered alone or in combination with atropine by intramuscular injection a slight protective effect against the oral LD_{50} was achieved (CGA-15324). The same type of combination was similarly effective aginst 3 repeated doses of the oral LD_{50} of CGA-15324; however, not against 5 repeated doses. When pam was administered alone or in combination wiht atropine by I.M. injection, no protective effect against the oral LD_{50} was achieved.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097797. Jan. 3, 1974, Batch No. P.1. Chicken, acute delayed neurotoxicity.

CGA-15324 and TOCP (triorthocresylphosphate) were tested by gavage on 12 (6M and 6F) and 8 (4M and 4F) domestic fowls, respectively - white leghorns. Animals were housed separately and observed a 2nd 21-day (42 days total). Animals treated with TOCP were observed 21 days.

Results

Mortality		Died With 7 Days	
Dose mg/kg	Test Material	Males	Females
21.7	CGA-15324	0	0
46.4		2	1
60.0		2	2
1000	TOCP	0	0
2150		0	0

The stated acute oral LD_{50} in fouls of both sexes was approximately 35 mg/kg.

About 2 hours after first treatment with CGA-15324, fouls in 46.4 and 60.0 groups showed salivation, asynchronism of extremities, curved position, apathy and ruffled feathers. No symptoms were observed as that time in animals treated with TOCP.

60 minutes before second CGA-15324 treatment with 21.7, or 46.4 mg/kg, animals were treated with atropine by I.M. route to the 5 surviving fouls.

20 hours after 2nd CGA-15324 treatment surviving animals showed salivation and asynchronism of extremities; those of the 46.4 mg/kg group died within 24 hours post treatment. No delayed neurotoxic symptoms were observed during 42 day observation period in animals treated with CGA-15324.

18 days after treatment the 4 fouls of the TOCP 2150 mg/kg group (2M and 2F), and 2M of the 1000 mg/kg group treated with TOCP showed a typical progressive ataxia and deterioration of reflexes (the + control).

No histopathology noted in CGA-15324 animals. TOCP birds showed swelling and fragmentation of myelin, several sites of demyelination.

Classification: Supplementary Data

(Animals should have been challenged with greater than an LD_{50} dose, part initially protected with atropine, to insure survival of at least 10 animals for behavioral, gross and microscopic examination.)

Tech. Chemical - I.B.T. No. 8580-11187, Acc. No. 097797. Chicken acute delayed neurotoxicity. July 25, 1978.

Test Material: CGA-15324 technical, purity 89.5%. ARS No. 2175/77, F1 No. 771423.

A. Acute Oral Toxicity

a. 7 groups of two adult hens each were treated with 10.0, 21.5, 31.6, 46.4, 68.1, 100.0, or 215.0 mg CGA-15324 technical/kg body weight. Doses administered by gavage in corn oil. Birds observed 14 days. Dying animals or those sacrificed at end of experiment were subjected to a complete necropsy examination.

b. Groups of 4 hens each were treated with 10.0, 14.7, 21.5, 31.6, 46.4, 68.1, or 100.0 mg CGA-15324 technical/kg body wt. administered in corn oil. Birds observed 14 days; animals that died or were sacrificed 14 days post treatment subjected to complete necropsy examination.

B. Neurotoxicity Study

4 groups of hens treated by gavage with single doses as follows: 10 birds untreated control, 15 birds positive control (TOCP), 40 birds T-I (30 mg/kg), and 50 birds T-II (treated at a calculated 45.7 mg/kg).

On test day 21 in each test group (due to an elevated mortality response at 30.0 and 45.7 mg/kg) all surviving birds (test) were redosed at a new estimated LD_{50} of 17.1 mg/kg. The new calculated LD_{50} was derived from the mortality response exhibited in the T-I and T-II test groups during the first 21 days of the experiment.

Control birds received corn oil. Positive control birds received a single oral dose of 500 mg/kg of ortho cresyl phosphate (TOCP).

Birds were observed daily for mortality neurotoxic signs.

Vertebral column sciatic nerve, brain examined for gross and histopathological study; including test and all vehicle and control birds, and redosed birds.

Results

A. Acute Oral Toxicity

a. All birds treated with 31.6, 46.4, 68.1, 100.0, or 215.0 mg/kg died. Birds dosed at 10.0 or 21.5 mg/kg survived and were sacrificed after 14 days observation.

b. Results of the second attempt to determine an LD_{50} follows:

mg/kg dose	dead/total	% dead
10.0	0/4	0
14.7	0/4	0
21.5	0/4	0
31.6	1/4	25
46.4	2/4	50
68.1	3/4	75
100.0	4/4	100

Necropsy examination of all dying birds and those sacrificed revealed no gross pathological changes that were treatment related.

An estimated LD_{50} determined from "6" above = 45.7 mg/kg.

B. Neurotoxicity Study

Body Weight Data - Positive control birds exhibited weight losses during 18 days on test. Mean body wt. gains were significantly depressed in group T-I (30 mg/kg), and group T-II (45.7 mg/kg) animals throughout the experiment.

Food Consumption - Food consumption depressions were noted in T-I and T-II test groups on day 7 and the positive control group on day 18. Food consumption for survivors in these groups was comparable to corn oil controls for remainder of the experiment.

Mortality - No deaths occurred in corn oil control group or positive control group animals up to sacrifice on day 18. Twenty-eight of 40 birds in the T-I group (30 mg/kg) died by day 6 during 1st 21 days of test. No deaths in this group following repeat dosing with 17.1 mg/kg. Forty-one of 50 birds died in the T-II group (45.7 mg/kg) by day 5 during the 1st 21 day period. One bird died in the T-II group following repeat dosing with 17.1 mg/kg during the 2nd 21 day period. The majority of birds found dead died within 24 hours of dosing.

All positive controls birds were sacrificed in extremis to obtain tissues on day 18; these birds displayed signs of delayed neurotoxicity by day 8 of test.

Gross Pathology - Remarkable lesions were confined to 3 test birds (all in group T-I, 30 mg/kg), and were not treatment related.

Histopathology - Histopathologic evaluation of specimens of brain, spinal c ord and sciatic nerve from birds treated with CGA-15324 revealed no treatment related lesions. Microscopic examination of 10 birds treated with corn oil revealed no treatment related lesions.

Treatment related changes were observed in all of the sciatic nerve and spinal c ord sections of the positive control birds. The sciatic nerve lesions consisted of focal to multifocal (demyelination) of the adjacent myelin sheath while the spinal c ord lesions consisted of focal axonal degeneration with or without demyelination.

No treatment related changes were observed in birds treated with technical CGA-15324.

Conclusions

Sufficient numbers of birds treated with CGA-15324 technical survived the 1st 21 day treatment to permit completion of the study; no delayed neurotoxicity was noted in birds treated with this chemical. Birds treated with corn oil vehicle also showed no acute or delayed neurotoxic symptoms. All positive control birds developed signs of delayed neurotoxicity and were sacrificed in extremis - all showed histopathological evidence of delayed neurotoxicity.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 31680, Acc. No. 097797.
Salmonella/Mammalian Microsome Mutagenicity Test. April 27, 1978

Salmonella typhimurium histidine auxotrophic bacteria strains employed were TA-98, TA-100, TA-1535, and TA-1537. The test was performed with the following concentrations of test material with and without microsomal activation: 5, 15, 45, 135, and 405 ug/0.1 ml. Test material was dissolved in DMSO; DMSO alone as used for the negative control.

Positive control experiments were carried out simultaneously with the following substances: 1) strain TA-1535: N-methyl-N'-nitro-N-nitroso-guanidine + phosphate buffer; 2) stain TA-1537: 9(5) aminoacridine hydrichloride monohydrates, and 25, 50, and 100 ug/1.0 ml DMSO; 3) strain TA-98: daunoblastin, 2.5, 5, and 10 ug/o.1 ml + phosphate buffer; 4) strain TA-100; 4-nitroquinoline-N-oxide, 0.0625, 0.125, and 0.25 ug/0.1 ml phosphate buffer - the activation mixture was tested with strain TA-1535 and cyclophosphamide, 100 and 250 ug/0.1 ml phosphate buffer.

Pour plates were encubated 48 hours. When colonies were counted, the arithmetic mean was calculated. Test substance was considered not to be mutagenic if the colony count in relation to the negative control was not doubled at any concentration.

Results

a. Without microsomal activation.

CGA-15324/ 0.1 ml	Strains of S. typhimurium			
	TA-98	TA-100	TA-1535	TA-1537
control	19	78	8	5
5 ug	15	75	8	5
15 ug	14	74	9	5
45 ug	17	70	8	5
135 ug	15	70	7	3
405 ug	18	69	10	5

The positive controls for each of the above strains were definitely positive.

b. With activation mixture (S9 fraction of liver from rats induced with Auroclor 1254).

CGA-15324/ 0.1 ml	Strains of S. typhimurium			
	TA-98	TA-100	TA-1535	TA-1537
control	28	82	19	10
5 ug	22	78	13	9
15 ug	29	82	17	11
45 ug	35	90	13	12
135 ug	21	87	16	9
405 ug	36	54	6	4

Positive control for microsomal activation (cyclophosphamide).

	(count)
control	25
100 ug/0.1 ml	241
250 ug/0.1 ml	251

Conclusion - CGA-15324 technical was not mutagenic when tested in the Salmonella/mammalian microsome mutagenicity test, with and without activation.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 327438, Acc. No. 097797. Mouse dominant lethal mutagenicity study. Batch No. 2.

CGA-15324 technical was administered orally in single doses by intubation to male mice which were then mated to untreated females over a period of 6 weeks. At the end of each week the females were replaced by new ones. Doses of 35 and 100 mg/kg given to the male mice.

This experiment was conducted to evaluate cytotoxic or mutagenic effects on male germinal cells as expressed by the loss of pre-implantation zygotes as well as the rate of deaths of post-implantation stages of embryonic development. An aqueous solution of carboxymethyl cellulose served as the vehicle (0.2 ml/kg boby wt.); tested in the control animals.

Each group consisted of 12 males, each of which was placed in a cage with 3 untreated females immediately after treating the males. At the end of one week the females were removed from the cage and replaced by another group of females; 6 mating periods spanned all stages of germ cell maturation.

The first week after administration of the test material, general condition, symptomatology and wt. gain were all checked. Females were autopsied on the 14th day of pregnancy; no. of live embryos and embryonic deaths listed. Uteri were placed in a solution of ammonium sulphide to detect sites of embryonic resorptions.

The t-test or Mann-Whitney's U tests were used to compare the totals of the no. of implantations; indicating possible pre-implantation losses. The totals of the number of mated and pregnant dams or embryonic deaths were compared with the aid of the X^2 test. Experimental data, particularly on the numbers of implantation and embryonic deaths were compared with spontaneous data of a cumulative of untreated controls observed over a longer period of time.

Conclusion

Females mated to males which has been treated with the compound did not differ significantly from the females mated to control males; neither in mating ratios nor in the number of implantations or embryonic deatns (resorptions). No evidence of dominant lethal effects was observed in the progeny of male mice treated with CGA-15324 technical chemical.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 22741900, Acc. No. 097797. Rat teratogenic evaluation. May 29, 1974, Batch No. mg 1.

One male/3 females were mated. 10, 30, or 60 mg/kg of CGA-15324 was administered to groups of pregnant rats from day 6 through day 15 of pregnancy; dams were autopsied at this time.

During the test, the general condition, weight gain and symptomatology were measured or observed for dams. Food consumption was noted.

Following assessment of dam organs, especially the ovaries and uteri, the foetuses were subjected to careful external inspection and the condition of their body ovifeces was checked. They were then individually weighed and submitted to the following procedures:

1. Assessment of body cavity sites (thorac, abdomen, pelvis).

2. Examination of viscera according to a slicing technique.

3. Skeletal assessment was performed as follows:

Group/dosage (mg/kg)	No. dams examined
10	25
30	20
60	27
carboxymethyl cellulose control	25
cumulative control	89

Results

Mean food consumption in dams was markedly reduced following the 60 mg/kg dose level. Food intake was slightly reduced at the 30 mg/kg dose level. No further signs of intolerability of the compound by the dams was noted. No significant deviations from the controls were found with regard to implantation ratio, embryolethality and foetal average weight.

Skeletal assessment of dams did not reveal any clear-cut differences from control groups.

Dystopia cordis in association with hypoplasia of lungs was detected in two foetuses of the 30 mg/kg group, in one foetus of the 60 mg/kg group and in one foetus of the control group.

Conclusion - No teratogenic effects from administration of CGA-15324 technical chemical to pregnant rats were observed.

Classification: Core-Guidelines Data

Tech. Chemical - Ciba-Geigy No. 3762, Acc. No. 097796. Dog 28-day feeding study. January 6, 1975

CGA-15324 technical, 94.8% pure O-(4-bromo-2-chlorophenyl)-O-ethyl-S-propyl phosphothioate.

000821

Procedure

CGA-15324 was diluted with polyethylene glycol 400 at 50%; pulverized dog food was then mixed and approximately 18% water was added before pelleting and air dyring. Control animals fed dog food alone. Fresh food prepared every two weeks. Concentration of CGA-15324 in food analytically determined to equal 90% of the added amount.

Dosage Levels

Group	No. Dogs M	No. Dogs F	Dietary Level ppm*	Food g/day M	Food g/day F	B. Weight kg M	B. Weight kg F	CGA-15324 mg/kg/day M	CGA-15324 mg/kg/day F
1	1	3	0	335	320	10.7	9.6	0	0
2	5 6	7 8	10	350	340	10.9	9.8	0.29	0.31
3	9 10	11 12	100	350	345	10.1	9.5	3.10	3.20
4	13 14	15 16	1000	345	325	10.7	8.7	29.0	33.0

*PPM CGA-15324 in diets was analytically confirmed.

Observations

Behavioral changes, treatment reactions, any ill health.

Unconsumed food from each dog collected and weighed in morning to determine dose levels.

Initial body weights of each dog, and weekly weighing recorded throughout test.

Ophthalmoscopic inspection performed prior to dosing and after one month.

Clinical Investigation

Hematology, blood chemistry and urinalysis performed on 16 (2 M/2 F/group) Beagles that were divided into 3 test and one control group. Complete test of clinical analyses carried out 4 days prior to substance administration and at 28 days. Cholinesterase activity assayed after 28 experimental days only.

Pathology

Macroscopical Examination - Dogs anesthetized and bled on the 28th day. Body weights determined. Individual weights of brain, heart, liver, kidneys, adrenals and gonads recorded. Organ to body weight and organ to brain weight ratios calculated.

Histopathological Examination - Tissues: pituitary, thyroid, thymus, heart, aorta, lung, stomach, small and large intestine, liver, pancreas, spleen, lymph node, adrenal, kidneys, urinary bladder, mammary glands and peripheral nerve, spinal cord and eyeball with optic nerve. Bone marrow samples from all treated control and test animals.

Results

Food consumption of treated animals was comparable to that of control animals. Female dogs in group 4 (1,000 ppm) lost body weight, whereas that of other treated dogs was not influenced by treatment when compared to controls.

Opthalmoscopic examinations did not reveal any ocular changes.

At experiment termination (after 28-days on test) all clinical laboratory investigations were considered within physiological limits.

At high and intermediate test concentrations there was dose-related and marked cholinesterase activity depression.

28th Test Day:

	Male		Female	
Dose (ppm)	Plas.	RBC	Plas.	RBC
0	68	77	61	92
	59	117	73	95
10	35	70	30	97
	36	64	23	74
100	25	35	16	36
	19	27	15	33
1000	16	15	14	17
	12	21	16	16
NOTE: % depression compared to controls for the 10 ppm dose.	Plas. 45	RBC 31	Plas. 60	RBC 8

As shown above, the 31% cholinesterase activity depressions for male dogs at the 10 ppm dose levels is significant; in fact, no NOEL for ChE activity was determined.

Urinalysis findings indicated no treatment related effects.

Aside from physiological variations, there were no differences in the absolute or relative organ weights between treated and control dogs..

Histopathologic Findings - The Ciba-Geigy report states that focal and dispersed pneumonic consolidation found in all treated animals was the result of parasitic activity. Since no pneumonic consolidation occurred in control animal tissues, it seems that these findings are in fact treatment related (10, 100, or 1000 ppm dose levels).

Conclusions

1) No significant treatment-related systemic macro or histopathological findings were noted. No behavioral changes, acute toxic treatment effects or ill health was noted.

2) At termination of test, marked ChE activity depression was found in all animals treated with 100 or 1000 ppm CGA-15324 technical. RBC ChE activity was depressed 8% or 31% at termination in female or male dogs respectively, treated with 10 ppm test material.

Classification: Core-Minimum Data

Tech. Chemical - Ciba-Geigy No. 2850, Acc. No. 097794. Guinea Pig dermal sensitization. May14, 1973

0.1 ml of a 1% solution of CGA-15324 technical was injected I.C. every other day into 10M and 10F guinea pigs for 9 injections, following an initial injection of 0.5 ml. After a rest period of 2 weeks, the animals were challenged with 0.5 ml of freshly prepared test solution.

Results

After each sensitizing injection, slight edema and erythema (1 mm high, 5 mm dia.) occurred. By 24 hours the findings had reduced to 0.2 mm height and 1 mm diameter. The challenge reaction was no more intense than those of sensitizing injections.

Conclusion

Technical CGA-15324 does not induce delayed type sensitivity in guinea pigs.

Classification: Supplementary Data (No + controls included)

Tech. Chemical - Ciba-Geigy No. 3647, Acc. No. 097794. Guinea Pig dermal sensitization. Batch No. mg 2.

Groups of 10M and 10F guinea pigs were given a series of 10 0.1 ml sensitizing injections I.C. every other day. Materials tested were a 1% saline solution of technical CGA-15324, 1% solution of dinitrochlorobenzene (+ control), and saline control (- control). A rest period of 2 weeks after the last sensitizing injection was followed by appropriate challenge injections of 0.1 ml each.

Results

Compound	No. + animals/ No. treated animals
saline	0/20
dinitrochlorobenzene (+ control)	20/20
CGA-15324	0/20

Conclusion

Technical CGA-15324 did not demonstrate any sensitizing potential in guinea pigs.

Classification: Core-Guidelines Data

Tech. Chemical - I.B.T. No. 611-05122-B, Acc. No. 097796. Dog subacute oral feeding "Final Report", previously reviewed by D. Ritter 2/2/77.

This report concerns a "Final Report" on IBT Study#611-05122-B (90-day subacute oral feeding study in beagle dogs), previously reviewed 2/2/77 by D. Ritter and listed by Ritter in a Tox. profile, 11/1/78.

The present "Final Report" is essentially the same as reviewed earlier by Ritter; its stated intended purpose is "our revised laboratory report" (for 611-05122-B).

Apparently, Ritter noted insignificant systemic effects of CGA-15324 in the 90-day dog study; however, cholinergic effects were noted at 20 and 200 ppm. Ritter also found that a definite NOEL was not determined at the 2 ppm lowest dose level tested.

The main thrust of the present report is to clarify the cholinergic effects found in this study.

Conclusions (Woodrow)

1) Plasma cholinesterase activity depression was demonstrated in both male and female dogs.

2) Marginal erythrocyte cholinesterase activity depression was found in male dogs only, after one month on test. At 2 and 3 months on test RBC ChE values were normal for male dogs.

3) Marginal brain cholinesterase activity depression was found for male dogs only after one month on test; ChE values for these animals returned to normal after 2 and 3 months treatment.

This reviewer agrees with D.L. Ritter's previous finding; a definite ChE NOEL was not established in this study.

Classification: Core-Minimum Data

Results

Compound	No. + animals/ No. treated animals
saline	0/20
dinitrochlorobenzene (+ control)	20/20
CGA-15324	0/20

Conclusion

Technical CGA-15324 did not demonstrate any sensitizing potential in guinea pigs.

Classification: Core-Guidelines Data

Tech. Chemical - I.B.T. No. 611-05122-B, Acc. No. 097796. Dog subacute oral feeding "Final Report", previously reviewed by D. Ritter 2/2/77.

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This reviewer agrees with D.L. Ritter's previous finding; a definite ChE NOEL was not established in this study.

Classification: Core-Minimum Data

Tech. Chemical - I.B.T. No. 8531-09996, Acc. No. 097796. Dog 90-day feeding study. May 9, 1977

Material Tested

CGA-15324 technical, 95.5% pure: O-(4-bromo-2-chlorophenyl)-O-ethyl-s-propyl phosphothioate

Procedure

Two groups of 4 male and 4 female Beagle dogs each were separately treated with 0 (group UC), or a diet that included a varying amount of CGA-15324 technical chemical (group T-I). Group T-I ppm test dietary levels were 0.1 ppm (days 1-14), 0.03 ppm (days 15-42), and 0.1 ppm (days 43-92).

Blood samples from all dogs were taken 3 times prior to start of the study and at 14-day intervals after testing began. These samples, obtained after 18 hours fasting, were analyzed for both plasma and erythrocyte cholinesterase activity. Brain cholinesterase activity was determined for each dog at study termination (92 days).

Results

RBC cholinesterase activity in Beagle dogs treated with CGA-15324 in diets remoles SH/ml/3 min (Treated animals only)

Group T-I diet level	Animal No. Sex	Pretest mean (AU-of 3)	14	28	42	56	70	84	92
	9-M*	6.7	-	-	-	-	-	-	-
0.1 (days 1-14)	10-M	4.3	3.6	3.1	3.7	3.8	3.5	3.1	3.2
0.03 (days 15-42)	11-M	5.5	5.0	5.5	4.9	5.3	5.1	4.7	4.7
	12-M	6.5	5.9	6.5	5.5	6.2	6.5	5.4	5.1
0.1 (days 43-92)	13-F	5.5	5.1	5.6	4.9	5.3	5.7	4.8	4.7
	14-F	7.0	6.6	6.8	6.0	6.5	6.6	5.7	5.6
	15-F	6.8	6.4	6.7	6.0	6.8	7.0	6.1	6.2
	16-F	4.0	3.8	4.7	4.4	4.3	4.6	3.6	3.7

*Animal removed from study (severe penile paraphimosis).

Plasma ChE Activity

All dogs showed a slightly depressed plasma ChE activity at 0.1 ppm for 14 days, followed by a return to normal values during the next 28 test days during which the dietary test level was reduced to 0.03 ppm. When the dietary level was again raised to 0.1 ppm for the last 50 days, no adverse effect on plasma ChE was noted.

Erythrocyte Cholinesterase Activity

Mean ChE values for control animals during the 4 day pre-treatment acclimation period was significantly higher than similar pre-treatment mean cholinesterase values for the T-I test group. For this reason, and for the sake of greater accuracy, the average of 3 pre-test RBC ChE values for each of the 8 animals in the test group, served as control values for comparison with T-I ChE values following treatment. An examination of the table above indicates:

1) Male No. 9-M was removed from the test which resulted in a 25% day drop in treated male test animals.

2) No. 10-M, RBC ChE reduced 16% by day 14 (0-1 ppm), 28% by day 28 (0.03 ppm), 14% by day 42 (0.03 ppm), 28% by day 84 (0.1 ppm) and finally 26% by day 92 (0.1 ppm).

3) Animal 12-M RBC ChE was reduced 22% by test termination data (0.1 ppm).

4) Animal 14-F, RBC ChE reduced 20% at test termination date (0.1 ppm).

Conclusions

1) Two of the 3 remaining male test animals showed definite RBC ChE cholinesterase depression (consider an approximate 20% reduction in RBC ChE activity to be significant).

2) One female test animal (14-F) showed a 20% reduction in RBC ChE activity at test termination; which is a marginal result.

3) Test dose levels should not have been varied. A wider span in dosage range should have been employed; for example, 0.03 ppm, 0.1 ppm, an 1.0 ppm. A fourth dose level of approximately 2.0 ppm should have been included to clarify earlier reports of a 2 ppm dietary NOEL.

4) It is apparent to this reviewer that no NOEL was established for erythrocyte cholinesterase activity in this experiment.

Classification: Supplementary Data

Tech. Chemical - I.B.T. No. 623-07924, Acc. No. 097798. Three-Generation Reproduction Study.

Test Material: CGA-15324 technical (FL 752154), 95.5% pure.

Three-generation repoduction study using CGA-15324 technical (FL 752154), 95.5% pure in albino rats. IBT (Industrial Bio-test Labs., 1810 Frontage Road, Northbrook, Illinois 6002.) Study No. 623-07924.

Ninety six weanling CD Charles River strain rats were divided into from 3 test and one control group of F_0 generation animals:

Group	Dietary Level (ppm)	Number of Animals Males	Females
C	0	8	16
T-I	0.2	8	16
T-II	1.0	8	16
T-III	20.0	8	16

Parental animals were allowed to reach maturity, mate and produce 2 litters. Eight males and 16 females from the second litter of each dietary group were retained at weaning as parental animals for the succeeding generations. Study terminated at weaning of F_3b litters.

Animals in all groups were maintained on their respective diets without interruption until sacrifice, which followed weaning of the second litters.

Fresh diets were prepared and offered weekly that incorporated a calculated weight of CGA technical in measured amounts of standard pulverized stock ration at dose levels of 0.2, 1.0, or 20.0 ppm. Control animals were fed basal ration only.

Initial body weights were recorded and each animal was weighed weekly until mating trails commenced. No additional body weight data was obtained until sacrifice of the parental generation, when final body weights were recorded.

Daily observations for mortality and abnormal behavioral reaction were made. Animals were observed for fertility, length of gestation and lactation performance. Fertility calculations: 1) fecundity index - of copulation resulting in pregnancy; 2) male fertility index - % males that had an opportunity to impregnate a fertile dam during mating trial that resulted in pregnancies; 3) female fertility index - % of pregnancies resulting from dams that were paired with fertile males.

All pups were examined for physical abnormalities at birth; numbers of viable and stillborn members of each litter were recorded. Survival at designated intervals during the lactation period was observed and a final examination for physical abnormalities was made at the weaning of each litter. Litters were reduced to 10 pups on the 4th day of the lactation period.

Mating trials were initiated when the parental animals were 100 days old. Females were caged in pairs and mated with a male from the same treatment group. Daily observations for copulation were made. Males were rotated within their dietary group at 10 day intervals until conception occurred or until each female has been paired with a maximum of 3 males.

Gross necropsies were performed on dead parental animals if autolysis was not advanced, tissues were removed for histopathology. After second litters were weaned, all surviving males and 8 females from each group were weighed, sacrificed, and subjected to gross pathologic examination. Weights of livers, kidneys, spleens, gonads, hearts, and brains were recorded.

At time of sacrifice animals were treated with CO_2 and exsanguinated. Complete microscopic studies were conducted upon 5M and 5F from the control and group T-III (20 ppm), and upon animals of the intermediate feeding levels which exhibited findings (gross) at time of sacrifice.

Gross Examination

Skin	Testes	Peripheral nerve
Heart	Ovaries	Eyes with optic nerve
Trachea	Prostate	Brain
Lungs	Seminal vesicles	Thymus
Liver	Uterus	Aorta
Pancreas	Vagina	Carcass
Esophagus	Pituitary glands	All gross lesions
Stomach	Adrenal "	
Intestinal tract	Salivary "	
Spleen	Thyroid "	
Lymph nodes	Parathyroid "	
Kidneys	Skeletal muscle	
Urinary bladder	Bone	

Histopathological Examination

Heart	Kidneys	Skeletal muscle
Trachea	Urinary bladder	Bone
Lung	Testes	Peripheral nerve
Liver	Ovary	Brain
Pancreas	Prostate	Seminal vesicles
Stomach	Uterus	Esophagus
Small Intestine	Pituitary gland	Spinal c ord
Ceacum	Adrenal "	
Colon	Salivary "	
Spleen	Thyroid "	
Lymph nodes	Parathyroid "	

Gross pathological examinations were conducted upon 10M and 10F weanlings of the F_3b litters of all groups. Complete histologic examinations were conducted upon the control and T-III weanlings only, unless tissue changes were observed.

Plasma, RBC, and brain cholinesterase activity determinations were conducted upon 5 parental M and 5 parental F of each test group following weaning of their second litters and upon 10M and 10F weanlings of each test group.

All population data and body weight data were analyzed statistically; first, a one way analyses of variance - significant effects detected by that method were further studied by Schiffe's Multiple Comparison Test, or Tukey's Multiple Comparison Test, depending on "N" values.

Statistical analyses were conducted on the absolute organ weights, and upon organ to body weight ratios and organ to brain weight ratios.

Results

No statistically significant differences in weight gains for treated F_0, F_1, and F_2 generations and corresponding control animals.

Several animals, both treated and control, died during the investigation; however, gross and histopathologic findings for these animals failed to reveal any treatment related causes for their deaths.

No unusual behavioral reactions occurred among parental animals which could be correlated with treatment.

Reduced RBC ChE activity was noted among M and F parental animals fed 20 ppm CGA-15324 throughout the study. Compared to controls, a statistically significant increase in plasma ChE activity was noted for F_0 females from the 0.2 or 1.0 treatment groups and for the F_1 males of the 1.0 ppm group. Reduced plasma ChE activity was statistically increased for F_1 parental animals given 1.0, or 20.0 ppm CGA-15324 when compared to controls. There were no differences in ChE activity in the F_3b pups. No other differences in ChE activity noted.

Mating indices were slightly reduced for F_1A & B litters at 20 ppm and 0.2 ppm. Parental animal mating indices were reduced for F_2A and B litters at 20 ppm; however, mating indices for the F_3A and B litters were unaffected at 20 ppm. F_2 parental animals fecundity index was reduced for F_2A litter at 1.0 ppm (not at 20 ppm).

For females the fertility index (parental animals) was reduced for the F_2A litter at 1.0 ppm; remaining F_2 litters at all doses were unaffected. The observed inconsistent parental animal reproductive responses are attributed to strain variance and not to reduced reproductive performance.

The number of stillborn pups in F_1A generation from parents fed 1.0 ppm and 20 ppm was significantly increased over control animals; this finding was not observed for F_1B pups. Numbers of stillborn pups from F_2A or B and F_3A or B litters were unaffected at all dose levels.

4 day survival indices for F_3A litter at 0.2 ppm, and 12 and 21 day survival indices for F_3A litters at 20 ppm were significantly reduced; however, comparable F_3A litters from parents at these dose levels were unaffected.

At 1 ppm dietary level F_1A females and F_1A males at 21 days exhibited slightly increased body weights, compared to controls. F_1A males and females at the 20 ppm dose level exhibited slightly increased body weights, F_1B 20 ppm malesor femaleswere unaffected, at 21 days. F_2 litters were unaffected by 21 days, while F_3B female at 1.0 or 20 ppm dosages showed slightly increased body weights.

NOTE: Test protocol stated that progeny body weights would also be measured at 0, 1, 4, and 12 days during treatment; however, this data was not available.

Coupled with the remaining reproduction data, the progeny 21 day results are considered sufficient. The inconsistent statisitcally significant weight differences (between litters) are attributed to rat strain differences.

Progeny development and behavior was not altered by dietary CGA-15324 treatment.

No differences between control or treated animals were found in progeny F_3bweanling plasma, RBC, or brain cholinesterase activity.

Parental animal/pathological findings

F_0 generation - 20 ppm female liver/body wt ratios slightly less than controls.

F_2 mean kidney weights for 20 ppm males slightly less than controls.

F_0 generation - 0.2, 1.0, and 20 ppm males - both gonad mean wts were significantly higher than control animals. 20 ppm male gonad/brain wt. ratios slightly higher than control animals.

NOTE: F_1 and F_3 generation gonad wts were comparable to control animals. Differences in male gonad wts were not considered treatment related.

Summary of non-neoplastic pathologic findings:

a) No treatment related findings (histopathologic) for animals sacrificed at end of experiment.

b) Post-mortem animals (results compared to controls)

	Dead		Findings
F_0 Males	T-II	T-III	
	2	4	0

(1 animal T-II, 1 animal T-III showed evidence of severe pneumonia, controls free of pneumonia.)

F_0 Females - 1 animal T-III had severe sinusoid congestion & pneumonia; 1 animal T-III showed acute bronchopneumonia, compared with controls.

F_1 Males - 2 animals T-I, 1 animal each for T-II and T-III; all showed evidence of severe bronchopneumonia.

F_2 Males - 4 animals C, 4 animals T-II, and 2 animals T-III all showed evidence of severe bronchopneumonia.

4 animals C, 5 animals T-II, and 2 animals T-III all showed histopathologic evidence of chronic murine pneumonia.

Conclusion:

There were no compound-related effects on body weight, mortality or any of the various reproductive indices which could be attributed to the test compound. Variations in certain mating fecundity on fertility were not consistent within a particular generation, nor were they dose-related. Based on a one-way analysis of variance which calculated the variance in the number of normal pups at day 21 per dam mated, no significant treatment effect was found. Pathological and histological results were unremarkable.

A consistent treatment effect on both male and female erythrocyte (RBC) cholinesterase values was noted in all parental generations at 20 ppm. A similar reduction in plasma cholinesterase was seen in females in all generations at 20 ppm.

The no-observed effect level for any reproductive parameter was the highest dose tested - 20 ppm, whereas 1 ppm was the NOEL for RBC and plasma cholinesterase inhibition.

Classification: Core-Minimum Data

000821

Formulation (CGA-15324 38% E.C.) - I.B.T. No. 8580-10426, Acc. No. 097797. Chicken delayed neurotoxicity study. June 6, 1977

This study was conducted in 2 phases in white leghorn hens maintained in 12 hours light and 12 hours dark.

A. Acute Oral Toxicity

a. ranging-finding - 3 groups of 2 birds each treated by gavage with 68.1, 215, or 316 mg CGA-15324 18% E.C./kg body wt. dissolved in corn oil. Birds observed daily for 14 days post treatment.

b. Groups of 4 hens each were treated with 46.4, 68.1, 100.0, 147.0, or 215.0 mg CGA-15324 38% E.C./kg body wt. dissolved in corn oil. Following treatment, birds observed 14 days. All animals were subjected to complete necropsy. Tissues showing abnormalities saved for histopathological examination.

c. Groups of 4 hens each treated in same manner with 68.1, 100.0, or 215.0 mg/kg and observed 14 days. Results of (b) and (c) experiments combined to construct an approximate acute oral LD_{50}.

B. Neurotoxicity Study

6 groups of hens treated as follows:

10 birds untreated control group, 10 birds positive control group (TOCP).

Dosage Level	mg/kg
15 birds - $LD_{50}/4$	29.2
15 birds $LD_{50}/2$	58.2
40 birds - 1 LD_{50}	117.0
30 birds - 2 LD_{50}	234.0

CGA-15324 38% dissolved in corn oil, prior to treatment by gavage. Birds fasted 16 hours before treating.

Approximately 1 hr. before treating, 15 mg/ml atropine sulfate was administered I.M. (breast muscle) at rate of 10 mg/kg to all birds in the 2 LD_{50} groups, and all birds in the control group. This procedure repeated on test day 21 with all birds surviving in these 2 groups. Positive control birds received a single oral dose of 500 mg/kg of TOCP on day 0.

Birds observed daily for mortality and possible neurotoxic signs for both 21 day observation periods. Body wts. recorded at 0, 21 and 42 days.

Neurotoxicity grading system:

0 = negative

1 = generalized weakness with or without intermittent ataxia

2 = slight continuous ataxia

3 = moderate to severe continuous ataxia

4 = bird unable to stand, paralysis of one or both legs

5 = birds unable to stand, paralysis of the legs and wings.

All birds that died during the study and all survivors at study termination received a thorough necropsy. Sciatic nerve spinal c ords and brains were examined for lesions. Tissue specimens selected for histological studies.

Results

A. Acute Oral Toxicity

Results of the study first phase (LD_{50} determination) showed an estimated acute oral LD_{50} to be 127.0 mg of CGA-15324 38% E.C./kg body wt. (X 0.38% of 127.0 = 48.6 mg technical chemical).

B. Neurotoxicity Study

All birds (30) treated with 234.0 mg/kg died within hours after treatment. Acute signs of cholinesterase inhibition (generalized weakness, legarthy, anorexia) were seen prior to death.

Twenty-two of 40 birds treated with 117.0 mg/kg died during the first few days of the study. Symptoms of acute cholinesterase inhibition similar to those described above (but somewhat less severe) were observed initially and complete recovery in the surviving birds was seen. One of the 15 birds treated with 58.5 mg/kg died and none of 15 birds treated with 29.2 mg/kg died during the experiment. Transient signs of cholinesterase inhibition were seen in these birds.

Twenty-one days following the initial dose, all surviving birds were again treated with the same CGA-15324 dose each had received initially. Twelve of 18 surviving birds treated with 117.0 mg/kg died during the first few hours post treatment. Symptoms of acute cholinesterase inhibition similar to those described after the first dose (on day 0) were observed initially, and complete recovery in surviving birds noted. One of the 14 surviving birds treated with a second dose of 58.5 mg/kg died and none of the 15 treated with 29.2 mg/kg died during the experiment. Treatment signs of cholinesterase inhibition were seen. No behavioral signs of neurotoxicity noted among any of the CGA-15324 38% treated birds during 42 days of test.

Positive control birds treated with a single dose of 500 mg/kg (TOCP) on day 0 exhibited behavioral signs of neurotoxicity by day 10 of the study. These birds were sacrificed in extremis on test day 18.

Gross and histopathologic studies of neural tissues from birds treated with CGA-15324 38% did not show any treatment-related changes (no delayed neurotoxicity). Treatment-related changes were evident in the spinal cord and sciatic nerves of all the positive control birds.

Classification: Core-Minimum Data

Study should have been conducted with technical chemical. Not enough birds were used to determine an accurate LD_{50}.

Chronic Toxicity

Formulation (CGA-15324 38% E.C.) - I.B.T. No. 622-07923, Acc. No. 097799. Mouse Chronic Feeding Study. November 27, 1978

Test Material: CGA-15324 technical was intended; CGA-15324 38% E.C. was actually used.

440 albino mice were divided into 4 groups of 55 animals per sex per group and fed daily dietary rations containing concentration of Curacron CGA-15324, 38% E.C. (FL - 740411) at the following levels:

Controls; 0 ppm - group T-I; .072 ppm - group T-II; 38 ppm and group T-III; and 114 ppm - group T-IV.

Feeding of the material was initiated when animals reached the age of 49 days.

Body weights were taken monthly starting at the 4th month of the study. Cholinesterase determinations were conducted in 5 male and 5 female mice from each group at the 12 month scheduled interval; and 10 male and 10 female mice per group at the termination of the study.

Fresh diets were prepared weekly. Analyses of the diet mixture was performed at week 75 of the study (first analyses) and at monthly intervals thereafter.

At 11-12 month of the study, feeder containers were changed to gravity feeders, in order to solve a sanitation problem.

Observations for mortality, abnormal behavior and gross physical abnormalities were performed daily. Such observations were recorded daily during the first six months of the study and twice per month thereafter.

Necropsies were performed in animals found dead. Microscopic examinations were performed on all animals sacrificed in extremis, animals sacrificed at the scheduled 12 month interval and all the animals sacrificed at termination.

Selected tissue samples representative of major organs and other tissues pertaining to the study, were stained for further histopathological examinations. Gross pathological examinations were performed in animals that died or were sacrificed during the study.

Results

Body Weight Changes: Unremarkable (similar to the ones for the controls). Changes noted at the 11-12 month, were due to the change of feeder containers.

Mortality: No treatment related mortalities were evident in this study. Dermal lesions not related to the administration of the compound developed in all the animals (both sexes, control and treated).

It is reported that at the two higher test dose levels the number of survivors was greater than the number of surviving control animals.

Histopathology: Examinations performed in animals that died or were sacrificed in a moribund condition and all animals sacrificed as scheduled revealed no treatment-related changes.

Conclusion

The administration of Curacron CGA-15324, 38% E.C. to male and female mice at dose levels of .072, 38 and 114 ppm active material incorporated in the diet, for 19 and 22 months, respectively, failed to produce histopathologic related occurrences that could be attributed to the administration of the compound. Cholinesterase activities were reduced at the two higher dose levels; 38 and 114 ppm (plasma, RBC and brain). Test animals fed 0.072 ppm (lowest dose tested) exhibited marginal reduction in RBC cholinesterase activity at the final bleeding. However, such a finding reveals meaningful effects, since the erythrocyte inhibition value was more than 20% lower than the value for the control animals, as shown in the following table.

Erythrocyte Cholinesterase inhibition in u-mol/ml/3 min (final reading at sacrifice).

	Control	T-I (0.072 ppm)	20% of control value
Male	9.3	6.6	1.86 = 7.44
Female	3.9	3.0	0.76 = 3.14

This study was conducted in the belief that the material was Technical Grade. It was later learned that the material was 38% EC.

The first analysis of the compound/diet mix was performed at 17 month of the study and 4 times only thereafter. We assume that mixing of the feed with the compound was done satisfactorily from the beginning of the study till the 17th month, since severe plasma cholinesterase inhibition was observed at the 12 month analysis interval, it indicates that test material was being incorporation into the diet in a homogenous way.

Neoplastic Findings: The neoplastic findings at 12th month interim sacrifice and at the final sacrifice were few and evenly distributed among all treated animals and the controls male and female.

Alveologenic Adenoma of the lungs were found in 2 male mice in the T-III group and one female in the T-I group at the 12th month interim sacrifice. At the final sacrifice a number of various neoplasms, evenly distributed among treated and control animals in the three test levels in both males and females, were found. Such neoplasms are believed to be of spontaneous occurrence in the strain of mice employed. Thus, the administration of the test material did not demonstrate oncogenic potential.

Hematology - No hematology studies were conducted.

NOTE: No RBC ChE. NOEL was determined.

Feeding Study - Supplementary Data - No hematology studies were performed.

Oncogenic Study - Minimum Data

This 2-year chronic and toxicity study was originally planned for CGA-15324 technical FL-752154 (purity 95.5%). It was later determined that the material used in the study was CGA-15324 technical FL-740411 (purity 38%). This error was well documented in the final validated report. Since diet calculations were made for the original test material (95.5% purity) and adjustments to 100% purity were made, the levels as reported in all tables of the final report (body weight, food consumption, etc.) are incorrect. Instead of 0.2, 1.0, 20, and 2000 ppm, the correct dietary levels are 0.08, 0.38, 7.6 and 76.0 ppm (Rats).

The animals employed in the study were Charles River Strain (CD) albino rats. Six hundred twenty rats (310 males and 310 females) were divided into experimental groups as shown below.

Procedure:

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Group	Dietary Level (ppm)*	Active Ingredients in diet (ppm)**	Number of Animals Male	Females
Control	0	0	60 + 5***	60 + 5***
T-I	0.2	0.08	60	60
T-II	1.0	0.38	60	60
T-III	20.0	7.6	60	60
T-IV	200.0	76.0	60 + 5***	60 + 5***

*Voluntary oral ingestion; ad libitum feeding of diets containing test material.

**Based on the 38% emulsifiable concentrate actually used.

***Animals used for recovery studies.

Body Weights - Each animal was weighed on test day 1, and weekly for 13 weeks, and monthly thereafter. Wts. were recorded and served as an index of growth.

Diet Preparation - Diets for test groups prepared by blending the appropriate amount of CGA-15324 38% E.C. (FL-740411) with standard rat ration. Fresh diets prepared each week.

Food Consumption - Food consumption data were collected from each of 10 rats per sex in each group weekly for 13 weeks, and at monthly intervals thereafter.

Diet samples were collected at study beginning and at 3, 6, 12, 18 and 24 month intervals. Samples were submitted to Ciba-Geigy for analysis.

Food Utilization - Food utilization was calculated on the basis of food intake and body weight gain for the first 6 months of the study.

Test Material Intake - The average body weights and food consumption data were used to calculate the test material intake (mg/kg) at 1.5, 3, 6, 13, 18 and 24 months of testing.

Mortality and Reactions - Routine checks for moribund or dead animals were conducted twice daily during the investigation. Observations for abnormal behavioral reactions and gross physical abnormalities were recorded weekly during the first 19 weeks of testing and monthly through month 15. Thereafter, these observations were recorded twice a month.

Hematologic, Clinical Blood Chemistry Studies, and Urinalysis - Blood and urine samples were collected individually from 20 rats/sex from the control and highest dietary level (200, actually 76 ppm) after 3, 6, 12, 13, 18 and 24 months of testing. Samples were analyzed for the following:

1. Hematologic Studies

   a. Total WBC
   b. RBC count
   c. Hemoglobin concentration
   d. Hematocrit value
   e. Differential WBC
   f. Platelet count
   g. Cell Indices (MCV, MCH MCHC)

2. Clinical Blood Chemistry Studies

   a. Serum alkalin phosphatase (SAP)
   b. Serum glutamic pyruvic transaminase activity (SAP)
   c. Blood urea nitrogen concentration (BUN).
   d. Fasting Blood and Glucose concentration.
   e. Total serum protein
   f. Total serum cholesterol
   g. Serum glutamic oxalacetic transaminase activity (SGOT)

3. Urinalysis

   a. Glucose concentration
   b. Albumin concentration
   c. pH
   d. Specific gravity
   e. Microscopic elements - crystals

Cholinesterase Determinations - Plasma and RBC cholinesterase determinations were conducted on 20 rats/sex from all groups after 3, 6, 12, 18 and 24 months of testing. Samples also collected from the designated recovery animals (5 males and 5 females of the control and high dose group) at 13, 14 and 16 months of testing. Recovery animals were to be removed from test diet following 12 months of consecutive feeding; however, these animals were inadvertantly maintained on test diet through Month 15. Animals were then removed from test diet at month 15 and allowed a 1-month recovery period. Blood samples for cholinesterase activity were collected prior to sacrifice at month 16.

Brain cholinesterase activity was determined at sacrifice for recovery animals (5 males and 5 females of the control and T-IV groups - 16 mo.) and on all remaining animals of each group at the final sacrifice.

Pathologic Studies - Complete gross necropsies were conducted on all animals found dead (unless autolyzed), all animals sacrificed in extremis, on the recovery animals and on all animals of each sex and group remaining at 24 months. At necropsy, representative tissues and organs were selected and fixed in 10 percent neutral buffered formalin. Organs weighed at the 16-month recovery sacrifice and 24-month final sacrifice included the adrenal glands, brain, gonads, heart, kidneys, liver, spleen and thyroid glands.

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Microscopic examinations were conducted on animals sacrificed in extremis, on all animals found dead, on recovery animals sacrificed at 16 months and on all animals remaining at 24 months.

Tissues, stained with Hematoxylin-Eosin, included the following:

Adrenal Glands
Aorta (thoracic)
Brain (cerebrum, cerebellum & pons)
Caecum
Colon
Epididymides
Esophagus
Eyes with Optic Nerve
Gonads (testes/ovaries)
Heart
Kidneys
Liver
Lung
Lymph Nodes (cervical & mesenteric)
Mammary Gland
Muscle (skeletal)
Pancreas
Peripheral Nerve (sciatic)
Pituitary Gland
Prostate Gland
Salivary Gland (sub-maxillary, sublingual, parotid)
Small Intestine (duodenum, jejunum, & ileum)
Spinal Cord
Spleen
Sternum (bone with marrow)
Stomach (cardia, fundis, & pylorus)
Thyroid Glands (with parathyroids attached)
Trachea
Urinary Bladder
Uterus

Gross pathologic examinations were conducted at Industrial Bio-Test Labs. However, tabulation of these findings and microscopic examination, evaluation and tabulation of all histopathologic findings are being conducted (at Ciba-Geigy's request) by Experimental Pathology Laboratories (EPL). The EPL report was submitted separately.

Statistical Analyses - Body wt. data, hematologic data, clinical blood chemistry data, cholinesterase data, and organ weight data were statistically analyzed. A one-way analysis of variance was conducted first. Significant effects disclosed by this analysis were further analyzed by either the Tukey's (equal population size) or the Scheffe's (unequal population size) Multiple Comparison Test. Organ to body weight and organ to brain weight ratios were statistically analyzed by the Kruskal-Wallis Statistic Test. Significant effects disclosed by this treatment were further analyzed by the Kruskal-Wallis Multiple Comparison Test. Statistically significant intergroup differences are indicated in the data tables; significant at the 95 percent confidence level ($p < 0.01$).

In appraising the data developed in this study, as well as historical information developed at IBT Laboratories for rats of the age and strain used have been considered. The presence of statistical significance did not lead per se to conclusions that test material-related effects occurred. Normal ranges for various parameters using Charles River Strain (CD) of rat have been established for a larger population of untreated rats than those used as controls in this study which provided additional values for final interpretation.

Results:

Body Weight and Weight Gain

Body weights and body weight gains among test animals were similar to those of controls. No treatment-related differences were noted.

Food Consumption

The amount of food consumed by rats fed CGA-15324 38% E.C. was similar to that eaten by control rats; no treatment-related effects.

Food Utilization

Some variation in food utilization occurred between various test groups; however, such differences were not treatment-related.

Test Material Intake

Analyses of test material consumed indicated values reasonably close to intended dosage rats. Periodic analyses for the lowest test group are shown below (group T-I). 0.2 ppm of technical chemical intended translates to 0.08 ppm (38% E.C. tested).

	Test Months					
Group	1 1/2	3	6	13	18	24
T-I						
Males	0.135	0.096	0.08	0.073	0.065	0.079
Females	0.148	0.115	0.111	0.091	0.079	0.095

Mortality and Reactions

Survival among treated rats was similar to that of the control animals.

Skin lesions were observed in both control and test animals, beginning during the second week of testing, which gradually cleared. The skin lesions were not treatment-related.

Hematologic Studies

No changes in the hematologic profile occurred in treated rats.

Clinical Blood Chemistry Studies

Clinical blood chemistry values for treated animals were similar to those found in control animals. No treatment-related effects.

Cholinesterase Determinations

A slight statistically significant decrease in erythrocyte cholinesterase activity was noted in T-I (0.076 ppm) females only at 3 months, and in males only at 12 months. Intergroup RBC values for the females at 3 months were variable and statistically different, which could account for the overall group depression in cholinesterase activity. This effect was only slight; no other incidence of depressed RBC ChE activity was noted throughout the 24 month experiment. It is believed that the two group RBC ChE T-I effects are artifactual in nature, not consistent, and not representative since males were affected in one instance and females in the other incidence. No RBC ChE activity inhibition was noted at the next highest T-II dose level (0.38 ppm).

Erythrocyte cholinesterase inhibition beginning at dose T-III (7.6 ppm) was fairly consistent in males or females or both males and females at each sampling period, except the 24 month sampling. More significant RBC ChE depression, apparently dose dependent, occurred at the T-IV (76.0 ppm) dose level.

One incidence of statistically significant plasma cholinesterase inhibition in males only occurred in the T-I (0.076 ppm) group at 12 months. No plasma ChE inhibition was noted in animals at the next highest T-II (0.38 ppm) group. Dose dependent plasma ChE depression apparently began in the T-III and T-IV animals.

Brain cholinesterase activity was measured at 16 and 24 months in control and T-IV group animals. Brain ChE activity determinations made on groups T-I, T-II, and T-III only at experiment termination (24 months). No brain ChE inhibition was noted for the T-I (0.076 ppm) males or females; however, brain ChE depression was noted in males of the T-II group, and apparent dose-dependent ChE (brain) inhibition in males and females of the T-III and T-IV groups.

It appears that a NOEL for cholinesterase activity determined in this 2-year rat study is 0.076 ppm, the lowest dose tested.

Urinalysis

Urinalyses were conducted on the control and T-IV test groups only (76.0 ppm). No treatment-related effects were observed in urine sample glucose, microscopic elements, pH or specific gravity.

Pathologic Studies

1. One-month recovery sacrifice animals (15 month treatment - one month recovery). No treatment-related alterations or statistical differences were noted in the organ weights and ratios among animals at the 1-month recovery sacrifice.

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2. Final sacrifice. Organ weights, organ to body weight ratios, and organ to brain weight ratios did not reveal any treatment-related alterations or statistical differences between control and treated animals at final sacrifice.

3. Gross and microscopic studies. Data regarding gross and microscopic findings accompanied by an interpretation of these data was included in a separate report prepared by Experimental Pathology Laboratories, Inc. (EPL). The tissues were processed for microscopic evaluation by IBT personnel. Microscopic evaluation of these tissues was initiated by Dr. Robl while at IBT, and was completed after he became employed at EPL.

Histopathological findings inidcated evidence of 1) myeloid hyperplasia, and 2) granulocytic leukemia.

Incidence of these two conditions that affect the hemopoietic system varied among the different control and test groups of animals. Such variation could not be directly correlated with cause. It was difficult to determine the influence of concurrent disease (respiratory tract, kidney, cardiovascular system, neoplasia, septicemia, and polyserositis) and any possible treatment-related effects upon the hemopoietic system through evaluation of tissue sections only. As a result, a more thorough analysis of the hemopoietic system by correlating all available hematologic and histopathologic data determined in this study has been done.

a) Granulocytic Leukemia

Histopathology data for all animals suffering from granulocytic leukemia were evaluated to determine if any of these animals were suffering from concurrent diseases listed above. One male (T-III), one control female, three T-I females, two T-III females and five T-IV females showed myeloid granulocytic leukemia; however, of these animals hematologic examinations were conducted only on the control female and four of the T-IV females.

Three of four of these animals had elevated leukocytes counts, and two of the four had reduced hemaglobin concentrations and hematocrit. These changes were somewhat consistent with a granulocytic leukemia; however, the available hematology data were too limited to sucessfully establish any trends. A comparison of histopathology in the other organs to that observed in bone marrow was made in an attemnt to establish the extent of the lesion; histopathology of liver, spleen and lung, infiltrates were noted in cervical and messenteric lymph nodes in many animals. Infiltrates were noted in all animals diagnosed with granulocytic leukemia at one or more sites.

Incidence of myeloid hyperplasia with regard to any associated changes in extramedullary hematopoiesis in the liver, spleen and adrenals was examined; therefore the association between extramedullary hematopoiesis (EMS) in these organs and granulocytic leukemia was evaluated. This comparison showed that of all animals having granulocytic leukemia, one liver in the T-IV group, and three spleens in the T-IV group were found to have EMH. None of these animals exhibited EMH of the adrenal glands. Thus, no association between EMH and granulocytic leukemia in the organs examined could be shown.

b) Myeloid Hyperplasia

The histopathology data indicated that the incidence of myeloid hyperplasia was increased in the T-I, T-II and T-III groups for both males and females, but was approximately the same for the control and T-IV groups. The incidence of myeloid hyperplasia was significant for both femur and sternum loci in the male and for the sternum locus in the female.

The myeloid or granulocytic cells in the bone marrow can be stimulated on become hyperplasia due to certain disease conditions. This ususally occurs in response to infections such as bronchiopneumonia, abcesses, skin ulcerations, uterine inflammation, or similar diseases. Since many of the animals in this study were affected with one or more of these diseases, the significance of the incidence of myeloid hyperplasia could not readily be established.

The relationship between hematologic parameters and histopathologic changes in othe organs and myeloid hyperplasia was examined. Endemic diseases (abcesses, skin ulceration, and uterine inflamnation) or lesions occurred in animlas diagnosed as having myeloid hyperplasia at a rate comparable to or less than the total incidence seen in each treatment group; with the exception of some lung abcesses, bronchial pneumonia and one incidence uterine inflammation. Despite the apparent association of lung abcesses with the occurrence of bronchial pneumonia, the incidence of pulmonary abcesses was not as high as the incidence of myeloid hyperplasia. The incidence of each disease or lesion and the total number of animals with diseases or lesion exhibiting myeloif hyperplasia were assessed; the incidence of endemic disease as represented by bronchial pneumonia, lung abcesses, skin ulcerations and uterine inflammation in animals affected by myeloid hyperplasia and fed the test material was not statistically different from the control animals.

The incidence of abnormal hematologic values for animals showing increased myeloid hyperplasias was examined. Even though the population of animals/sex/group were limited, the hematologic parameters were not significant for all animals diagnosed as having myeloid hyperplasia.

The incidence of extramedullary hematopoiesis in the liver, spleen, adrenal and lung of animals diagnosed as having myeloid hyperplasia was reviewed. Only in the case of the incidence of extramedullary hematopoiesis in the spleen of T-I females was statistically significant difference noted in regard to the incidence in the population in a given test group versus the population with myeloid hyperplasia. Therefore, it was not possible to establish whether extramedullary hematopoiesis in the organs examined is a result of myeloid hyperplasia or a condition caused by other factors.

A further examination of the relationship between extramedullary hematopoiesis and myeloid hyperplasia was made by comparing the incidence of extramedullary hematopoiesis for the spleen, liver, adrenal and lung combined with that of myeloid hyperplasia. The results of this analysis indicate the incidence of extramedullary hematopoiesis is not different among groups for those animals having myeloid hyperplasia.

In addition to evaluating hematologic and histopathologic findings, the spleen and organ weights and their respective organ-to-brain weight ratios for those animals sa[illegible] at 24 months having myeloid hyperplasia were examined [illegible] spleen adn related organ-to-brain ratios were not different [illegible] oup population mean in all animals diagnosed as having myeloid [illegible] ia.

No relationship between several hematologic parameters or histopathologic lesions of the leukopoietic system and both granulocytic leukemia and myeloid hyperplasia could be established. In addition, no consistent trends between endemic disease states and these lesions of bone marrow could be established. Animals diagnosed with granulocytic leukemia and myeloid hyperplasia also suffered from pathologic conditions in other organs of the leukopoietic system and endemic disease and lesions, but no specific relationships could be established.

The type of tremors observed in this study are not uncommon in this strain of rats. The incidence of tumors was higher among females when compared to the males. This is not considered unusual since pituitary and mammary gland tumors are much more prevalent among females. In most instances the incidence of tumors was similar among control and test animals.

Conclusions

1) Curacron was determined not to be oncogenic in this study.

2) No significant systemic toxic effects were determined in this 2-year chronic rat feeding study; however, Curacron was shown to produce strong cholinergic effects. A slight decrease in RBC ChE activity was noted in T-I (0.076 ppm) females only, and one instance of plasma ChE depression in T-I males occurred. No RBC or plasma ChE inhibition occurred at the next highest dose level, T-II (0.38 ppm); whereas dose dependent ChE inhibition was apparent at higher dose levels. It was concluded that the slight ChE inhibition observed at the T-I dose level (plasma and RBC) was artifactual in nature and not significant.

   No brain ChE inhibition was noted at the lowest dose tested (0.076 ppm), but was observed at the next highest dose level. Therefore a brain ChE NOEL was determined to be 0.076 ppm.

   Using a 10 fold safety factor, an ADI for Curacron would be 0.0004 mg/kg BW. A corresponding MPI for 60 kg adult would be 0.024 mg/day.

3) Toxicity studies with Curacron indicated no systemic toxicity; however, significant cholinergic effects were found.

4) Based on a two-year rat chronic feeding study, a brain cholinesterase inhibition NOEL was 0.08 ppm, and corresponding maximum permissible intake (MPI) is 0.024 mg/day.

5) The theoretical maximum residue contribution (TMRC) based on requested tolerances of 0.702 mg/1.5 kg daily diet, exceeds the MPI by a factor of 29.

6) Final validation of the IBT studies included in this report must be completed prior to use of such studies to support requested tolerances.

7) Residue levels have not been defined.

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F. Evaluation of Requested Tolerances:

PP#8F2057, PP#8H5177 (100-L00, 100-L0I), and PP#9G2234, PP#9H5231 (100-EUP-AT)

1. Two 90-day subchronic oral feeding studies would ordinarily be sufficient to make an EUP hazard assessment; however, no NOEL was determined for Curacron (ChE inhibition) in the studies reviewed.

   A 90-day dog IBT subchronic study using the technical chemical showed plasma ChE depression in males of 60, 45, and 52% at 1, 2, and 3 months respectively, of test; and ChE activity depression of 57, 52, and 58% in female dogs during the same test periods at the LDT (lowest dose tested) of 2.0 ppm. RBC and brain ChE inhibition during the same test periods was marginal.

   An IBT mouse chronic feeding study using 38% E.C. CGA-15324 (Curacron) showed significant RBC ChE inhibition at 0.072 ppm, the lowest dose tested.

   Females fed 114 ppm in the mouse experiment showed statistically significant RBC and brain ChE depression at 12 months, while males did not. Male mice fed 114 ppm and females fed 38 ppm exhibited reduced (not statistically significant) RBC ChE values at 12 months.

   Test animals fed 30 ppm or more had significantly reduced RBC ChE activity at test termination. At termination (22 months), no reduction in plasma or brain ChE activity at the lowest dose tested (0.072 ppm) was found; however, 29% and 23% reductions in RBC ChE activity in male and female mice, respectively, was shown.

   Thus, neither the 90-day dog subchronic, nor the 22-month mouse chronic feeding studies could be used to make a hazard assessment for 100-EUP-AT.

2. A two-year IBT rat chronic feeding study using 38% CGA-15324 (Curacron) indicated a brain ChE activity NOEL of 0.076 (0.08) ppm:

   A slight statistically significant decrease in ChE activity was noted in 0.076 ppm females only at 3 months and in males only at 12 months (0.076 ppm was lowest dose tested). It was believed that the two values determined were artifactual and not consistent; since males were affected in one instance and females in the other instance.

   No RBC ChE inhibition was noted at the next highest dose tested (0.38 ppm). RBC ChE inhibition was fairly consistent in both males and females beginning at the next highest dose of 7.6 ppm, and apparently dose dependent RBC ChE inhibition occurred at the 76.0 ppm dose level.

   One incidence of statistically significant plasma ChE inhibition in males only occurred in the 0.076 (LDT) group at 12 months. No plasma ChE inhibition was noted in animals at the next highest (0.38 ppm) group. Dose dependent plasma ChE depression apparently began in the 7.6 and 76.0 ppm animals.

Brain ChE activity was measured at 16 and 24 months in control and 76.0 ppm animals. Brain ChE activity was measured for 0.076, 0.38, and 7.6 ppm animals at experiment termination only. No brain ChE inhibition was noted for the 0.076 ppm males or females; however, brain ChE depression was noted in males of the next highest (LEL) of 0.38 ppm. Apparent dose dependent brain ChE inhibition in males and females occurred in the 7.6 and 76.0 ppm dose groups.

Therefore, it appears that a NOEL for brain cholinesterase activity was determined to be 0.076 ppm, the lowest dose tested while the LEL was considered to be the next higher dose, 0.38 ppm.

Based on a brain ChE activity NOEL of 0.076 ppm (2-year rat chronic feeding study - this report) and using a 10 fold safety factor, an ADI for Curacron would be 0.0004 mg/kg B.W.. A corresponding MPI for 60 kg adult would be 0.024 mg/day.

Calculation of theoretical maximum residue contribution (TMRC) to the human diet:

PP#8F2057, PP#8H5177 (100-L00, 100-L0I), Curacron - growing cotton.

PP#9G2234, PP#9H5231 (100-EUP-AT), Curacron on soybeans.

55 ppm - soybean fodder (straw) would not directly contribute to the human diet. However, the 0.05 ppm secondary residues requested for meat and meat by-products which could be directly affected by the 55 ppm soybean fodder request, cannot be assessed at present according to Residue Chemistry Branch, due to lack of adequate animal feeding studies.

In addition, 2.0 ppm soybean meal and hulls would not contribute to the human diet.

Pending final approval of 0.05 ppm, secondary residues in meat and meat by-products, a TMRC would show:

1.0 ppm soybean (1.0 mg/kg soybeans X 0.0092, fraction in diet X 1.5 kg diet) = 0.014 mg/day.

0.05 ppm, eggs, meat, fat, meat by-products of cattle, goats, hogs, horses, poultry and sheep (0.05 mg/kg meat X 0.13, fraction in diet, X 1.5 kg diet) = 0.009 mg/day.

0.01 ppm, milk (0.01 mg/kg milk X 0.286, fraction in diet X 1.5 kg diet) = 0.0043 mg/day.

3.0 ppm, cottonseed (3.0 mg/kg cottonseed X 0.15, fraction in diet X 1.5 kg diet) = 0.675 mg/day.

Total TMRC = 0.702 mg/1.5 kg daily diet. The calculated MPI = 0.024 mg/day

Conclusions

1) Pending approval of the requested 0.05 ppm residues for eggs, meat and meat by-products by Residue Chemistry Branch, the total TMRC (0.702) exceeds the calculated MPI of 0.024 by a factor of 29.

2) A second oncogenic study should be performed.

3) Final validation of the IBT studies included in this report must be completed prior to use of such studies to support appropriate requests.