

000179

ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Date: November 16, 1972
Reply to: Request for residue tolerance for the insecticide Chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate] and its metabolite 3,5,6-trichloro-2-pyridinol in or on fat and (on fat basis) meat and meat byproducts of cattle at 1 ppm, meat, fat and meat byproducts of turkeys at 0.2 ppm, and peaches and field corn (grain, green forage, and fodder) at 0.05 ppm (negligible residues).
Subject: To:

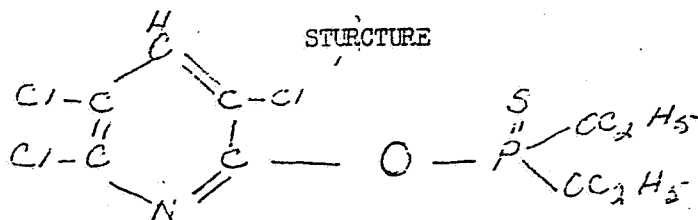
Mr. Lee TerBush, Acting Chief
Coordination Branch
Registration Division

Pesticide Petition No. 3F1306

Dow Chemical USA
Midland, Michigan 48640

Related Petitions - None

Other Names - Dursban, Lorsban, DOWCO 179, ENT 27311



O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate

FORMULATIONS

I. Chlorpyrifos Technical

O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl)
phosphorothioate

min. 94%

Impurities

max. 6%



10642

Impurities Information

II. Dursban 25W Insecticide

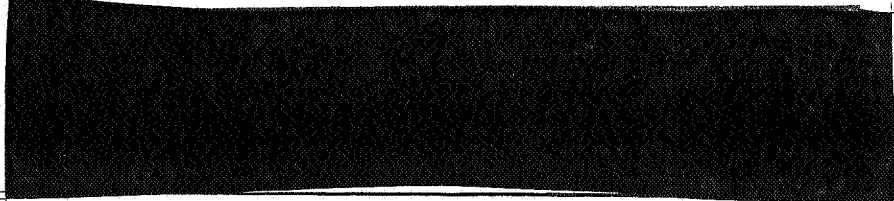
Active Ingredient:

Chlorpyrifos

25.0%

Inert Ingredients:

75.0%



III. Dursban 24E Insecticide Cattle Dip and Spray

Active Ingredient:

Chlorpyrifos

23.7%

Inert Ingredients:

76.3%



IV. Lorsban 2E Insecticide

Active Ingredient:

Chlorpyrifos

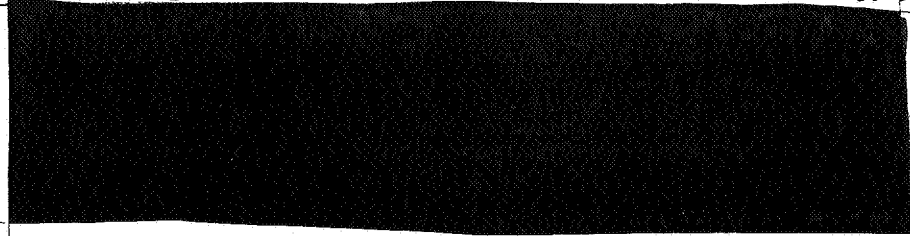
22.4%

Xylene

42.1%

Inert Ingredients:

35.5%



SECRET INGREDIENT INFORMATION IS NOT INCLUDED

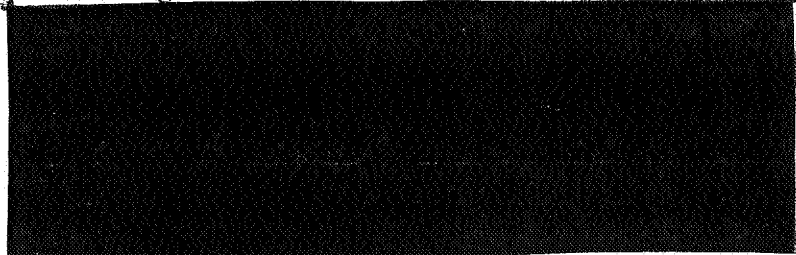
V. Lorsban 4E Insecticide

Active Ingredients:

Chlorpyrifos
Xylene

41.2%
29.5%
29.3%

Inert Ingredients:



VI. Lorsban 10G Granular Insecticide

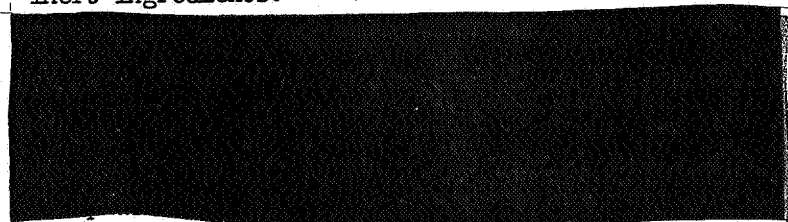
Active Ingredient:

Chlorpyrifos

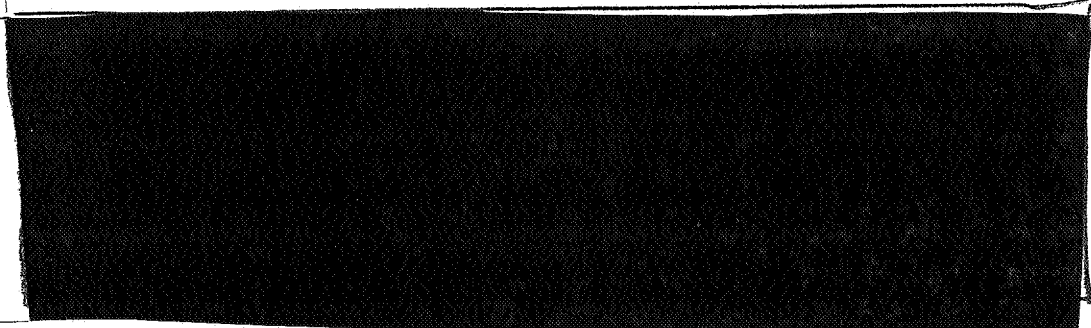
10%

Inert Ingredients:

90%



- * Approved for use under 40 CFR 180.1001(d)
- ** Approved for use under 40 CFR 180.1001(c)
- *** Approved for use under 40 CFR 180.1001(e)
- **** Currently under scientific review



INERT INGREDIENT INFORMATION IS NOT INCLUDED

The first two constituents are approved for use on animals under 40 CFR 180.1091(e) but not the third.

USES

I. Dursban 25W Insecticide will be applied to the soil in turkey pens but not directly to the turkeys (for control of chiggers).

II. Dursban 24E Insecticide will be applied topically (and used as a dip) for the control of ticks and other ectoparasites on cattle.

III. Lorsban 2E and Lorsban 4E Insecticide will be applied to the trunks of peach trees for the control of the peach tree borer.

IV. Lorsban 10G Granular Insecticide will be applied to the corn seed row at planting for control of corn rootworms.

TOXICOLOGICAL EVALUATION

I. Acute Oral Toxicity

Species	Sex	Material in Which Chlorpyrifos Administered	LD50 mg/kg body weight (95% confidence limits)
Rat	M	Corn oil	163 (97-276) ✓
		Corn oil	245 (219-273)
		Corn oil	118 (77-181)
		Dursban 24E Dose 0.5/100 112.5	713
		Peanut oil	155 (132-181)
	F	Dursban 25W	410
		Water-gum acacia	151
		Corn oil	137 (97-188)
		Dursban 25W	200
		Dursban 24E	356 (243-523)
Guinea pig	M	Corn oil	82 (69-97)
Rabbit (white)	M&F	Corn oil	504 (299-850) ✓
Mouse (white)	M	Water-gum tragacanth	1000-2000
Mouse (white-footed)	M		102 ± 8.3*
Chick (leghorn)	M	Corn oil	62 ✓
Chicken (leghorn)	M	Capsule	32 (14-72) ✓
		Capsule	25.4 (20.2-30.9)
		Capsule Dose 0.5/100	31.6
Chicken (white rock)	M&F	Capsule Dose 0.5/100	50-63
Turkey (poult)	M&F	Capsule	32-63
(4 wk old)		Capsule	30
		Capsule	<20
(12 wk old)			
Duckling (mallard)	M&F	Water-gum acacia	167 (78.3-357)
Duck (mallard)	M&F	Capsule	75.6 (35.4-161)
Pheasant	M	Capsule	8.41 (2.77-25.5)
Chukar	M	Capsule	17.7
		Capsule	61.1 (47.5-78.6)
		Capsule	60.7 (43.8-84.1)

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I. Acute Oral Toxicity (con't.)

<u>Species</u>	<u>Sex</u>	<u>Material in Which Chlorpyrifos Administered</u>	<u>LD50 mg/kg body weight (95% confidence limits)</u>
Goat	F	Capsule	500-1000
*24 hour LD50			

II. Acute Oral Toxicity - cattle and sheep

<u>Species</u>	<u>No.</u>	<u>Dosage(mg/kg)</u>	<u>Formulation</u>	<u>Duration(days)</u>	<u>Deaths</u>
Calf	2	125	KM*	4	1
	2	100	KM	5	2
	1	75	Technical	5	
	2	100	Technical	6	
	1	500	Technical	3	
	1	25	2 lb/gal E.C.	3	
	1	2.5	2 lb/gal E.C.	3	
	1	5.0	2 lb/gal E.C.	3	0
Cattle	3	2.5	2 lb/gal E.C.	3	0
Sheep	3	300	CO*	6	1
	1	250	CMEK*	-	0
	5	300	CMEK	6	2
	2	400	CMEK	9	2
	2	400	KM	-	0
	15	300	CMEK	7	5
	15	300	SM*	7	1
	15	300	KM	7	3
	15	300	CO	7	12
Sheep (good condition)	25	250	KM	6	1
Sheep (poor condition)	25	250	KM	14	2
Sheep (good condition)	25	200	KM	-	0
	25	200	KM	6	1
	4	200	25% WP	3	1
	5	150	25% WP	-	0
	5	100	25% WP	-	0
	5	50	25% WP	-	0

*KM = kaolin + methyl cellulose in water

CO = castor oil + ethanol (80:20)

CMEK = castor oil + methocel + ethanol + kaolin

SM = silica gel + methyl cellulose in water

Signs of toxicity included diarrhea, excessive salivation, labored and rapid respiration, fasciculations, stiffness in hindquarters, difficulty in standing, and anorexia due to loss of tongue control.

III. Acute Cholinesterase Activity Studies

- A. Three mongrel dogs and three rhesus monkeys were orally dosed with an equimolar dose of 10 micromoles/kg Dowco 179. Heparinized blood samples were taken at 4, 8, and 24 hours and then daily for 7 days. ChE activity was determined by the manometric method.

Species	Type ChE	% of Baseline Activity During Control Period			
		4 hr.	8 hr.	24 hr.	48 hr.
Dog	RBC	79	84	75	84
	Plasma	16	31	77	67
Monkey	RBC	60	66	60	82
	Plasma	6	8	14	30

- B. Male rats were orally dosed with one of the following 4 dosage levels of Dursban:

Dose (g/kg)	Cholinesterase (units/ml)*	
	RBC	Serum
0.063	18.5	3.7
0.126	21.4	0.1
0.252	18.7	0.3
0.50	20.9	0.4
Control	43.9	8.3

*Method not given.

IV. Acute Dermal Toxicity

Species	No.	Formulation	Results
Rabbit	3	Undiluted (intact skin)	0 died
	3	Undiluted (abraded skin)	2 died
	3	50% soln in Dowanol DFM (intact skin)	2 died
	3	50% soln in Dowanol DFM (abraded skin)	2 died
	4	Dursban 2LE	* LD ₅₀ 3360 (2000-5650) mg/kg
	12	Dursban 25W	LD ₅₀ 2.83 g/kg

(Table cont. on Page 7)

IV. Acute Dermal Toxicity (cont.)

<u>Species</u>	<u>No.</u>	<u>Formulation</u>	<u>Results</u>
Rats	40	Bursban	LD ₅₀ 202 (176-232) mg/kg
Turkey (8 wk old)	5/grp.	4, 8, 16 & 32 lb/A	No ill effects
	2	0.5% spray-saturated	2 died in 6 hours
	2	0.5% spray-mist on heads and backs	No ill effects
	2	0.25% spray-saturated	2 died in 8 hours
	2	0.25% spray-mist on heads and backs	No ill effects
	2	2 gm 25% WP	2 died in 8 hours
Turkey (10 wk old)	2	3750 mg 25% WP	2 died in 14 hours
	2	1000 mg 25% WP	2 died in 5 hours
	2	500 mg 25% WP	2 died in 4 hours
	2	100 mg 25% WP	1 died in 7 hours
	2	0.5% spray-wetheads & backs	2 died in 6 hours
	2	0.25% spray-wetheads & backs	2 died in 24 hours
Turkey (18 wk old)	5/grp.	4, 8, 16, & 32 lb/A	1 32 lb/A bird became ill
Turkey (20 wk old)	1	25 mg/kg of 25% WP	1 died in 24 hours
	4	20 mg/kg of 25% WP	3 died in 24 hours
	4	15 mg/kg of 25% WP	No ill effects
Calf	1	0.25%	ChE depression
	1	0.5%	Poisoned and survived; ChE depression
Cattle	44	0.1%	ChE depression
	8	0.05%	ChE depression
	16	0.01%	ChE depression
	8	0.005%	ChE depression
	8	0.025%, spray	No ill effects
	8	0.025%, dip	ChE depression

V. Eye Irritation

The results of an eye irritation study using undiluted Chlorpyrifos Technical in the conjunctival sac of rabbits were reported. Very slight to slight pain upon direct contact was reported and slight conjunctival redness subsided within 24 hours in two cases and within 48 hours in a third case. The other three rabbits sustained slight conjunctival redness after 7 days. The results in 6 rabbits in which the eyes were washed with water after application were essentially the same.

(Undiluted Dursban 25 W applied directly to rabbits eyes produced slight to moderate conjunctival erythema and slight transient corneal haziness. Recovery was essentially complete 48 hours post-instillation.)

- * Instillation of Dursban 24E into the eyes of 6 rabbits caused conjunctival redness and chemosis, iritis, and corneal injury resulting in a positive response in all of the treated eyes.

VI. Acute Toxicity (Only results submitted)

<u>Species</u>	<u>Route</u>	<u>Diluent</u>	<u>LD₅₀ (mg/kg)</u>
Mice	ip	Propylene glycol	30.8
Rats	sc	peanut oil	>200

VII. Skin Irritation

Three rabbits each were subjected to 10 (intact skin) or 3 (abraded skin) applications of undiluted dry Chlorpyrifos while 2 rabbits/group were treated with the undiluted wet material for similar periods. Slight hyperemia was first noted which developed into a slight burn in about half the animals during the second week. Slight exfoliation was observed during the second week. The skin healed normally leaving no scar in 21 days.

(Undiluted Dursban 25W when applied to intact and abraded rabbit skin on a prolonged repeated (3 weeks) confined basis produced essentially no discernible signs of irritation.)

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Application and confinement of Dursban 24E to the intact and abraded skin of 6 rabbits resulted in moderate to severe erythema on all exposed skin areas with a slight necrosis on 1 of the intact areas and 5 of the abraded areas. The edematous response was moderated or severe on all exposed skin areas except for one which displayed a slight edema. Skin reactions to continuous contact over a period of 6 hours, evaluated on intact and abraded dorsal skin of 3 rabbits on a skin irritation patch test, were slight erythema in 10 minutes, slight edema in 30 to 60 minutes and slight necrosis in 1.5 to 3.5 hours.

VIII. Inhalation Toxicity

Exposure of 10 rats/sex to the vapors of Dursban 24E at a concentration of 5 mg/l for 1 hour resulted in 100% survival and no more than slight lachrymation, slight nasal discharge, and some slight respiratory difficulty (gasping) during the exposure.

IX. Short-Term Feeding Studies

A. Chicks - 2 weeks

Twenty chicks/dosage level were fed diets containing 0, 200, 400, or 800 ppm Dursban for 2 weeks. The results were reported as follows:

Concentration (ppm)	Blood ChE inhibition %		Mortality %		Mean weight:gain ratio	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
800	90	-	65	100	0.79*	-
400	90	78	10	45	0.98*	1.27*
200	83	72	0	0	1.46*	1.46
0	-	-	0	0	2.14	1.52

*Significantly lower than control group, $P < 0.01$.

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B. Bullfrogs - 3 days

Two captive male bullfrogs received oral dosages of Dursban and survived without marked effect. One received 100 mg/kg while the other received 100, 200, and 400 mg/kg on successive days.

C. Wild fowl - 5 days

Dursban was fed to 5-7 day old mallard ducklings or bobwhite quail for 5 days. Following 3 more days for observation, the LC₅₀-3 was computed:

Mallard ducklings	LC ₅₀ 361 ppm
	LC ₅₀ 133 ppm
Bobwhite quail	LC ₅₀ 721 ppm

With Dursban, the growth and feed consumption of Mallard ducklings were adversely affected at dietary levels of 90 ppm. Dursban produced marginal inhibition of whole blood ChE at 1 ppm and marked inhibition of brain enzyme at 90 ppm.

D. Monkeys - 3 days

Two rhesus monkeys were given doses of 2 mg/kg of Dursban by stomach tube for 3 consecutive days. Blood samples were taken at 0, 24, 48, and 72 hours and ChE activity was measured by the pH stat and manometric methods.

No change in behavior or sign of parasympathetic stimulation was observed in either monkey. A sharp decrease in plasma ChE activity was observed 24 hr. after the initial dose with slightly greater reductions after the second and third doses. RBC ChE activity decreased very little following the first dose with greater reductions following the second and third doses.

X. Antidote Activity

- A. Six calves were given an oral dose of 500 mg/kg of Dursban. One calf, which served as the control and was not treated with antidotes, died within 72 hrs. Two calves were treated with 20 mg atropine/100 lb, two with 1 gm of Protopam Chloride, and one with a combination of the above (20 mg atropine/100 lb and 1 gm of Protopam Chloride). Treatments were administered 2-3 times/day. The 5 treated calves responded temporarily, but apparently the dose was too low for permanent regression of symptoms. The calves died or were killed because recovery was considered impossible between 5 and 8 days after administration.

of Dursban.

- B. Four calves were given an oral dose of 250 mg/kg technical Dursban. One calf was used as a control and was not treated with 20 mg atropine/100 lb until 48 hours after dosing with Dursban. Response to atropine at this time was nil. Treatment with atropine was started on the other three calves with 20 mg atropine/100 lb b.i.d. at 22 hours after dosing with Dursban. One calf recovered and the other 2 survived until the 11th and 12th days.

XI. Subacute Toxicity

- A. Results of 90-Day Dietary Feeding Studies of O,O-Diethyl O-3,5,6-Trichloro-2-Pyridyl Phosphorothioate in Rats (Dow; April 5, 1971) 11246

1. Procedure

Groups of 10 rats/sex/dosage level were fed diets containing 0, ~~0.01~~, 0.03, 0.01, 0.003, or 0.001% Dursban (98% pure) for 90 days. (The 0.1% group was eliminated from the study after 4 weeks.) Additional groups of 6 rats/sex/dosage level were fed these same diets to be used for interim ChE determinations.

The rats were weighed twice weekly for the first 28 days and once a week thereafter. Records were kept of mortality and food consumption was recorded for the first month. Plasma, RBC, and brain ChE activity were determined (using Technican Auto-Analyzer) on 3 rats/sex/level after 14 and 41 days. Five rats/sex were sampled after 90 days. Terminal hematological values (hematocrit, hemoglobin, and total and differential WBC) were obtained from 5 F rats in the 0, 0.03 and 0.01% groups. Samples of blood serum were obtained from members of both sexes in all groups for determination of urea nitrogen content and SAP activity.

The lungs, heart, liver, kidney, spleen, and testes were removed and weighed. Portions of each organ, as well as brain, thyroid, parathyroid, adrenal, thymus, stomach, small and large intestine, pancreas, urinary bladder, ovary, prostate, and uterus were examined microscopically (H&E stained).

2. Results

The 0.1% rats were discontinued after 4 weeks due to dramatic weight loss and high mortality. Tremors, moist bloody noses, circling and backing, emaciation, and ulceration of the cornea and around the nostrils were observed in these rats. After 2 weeks, ChE activity in the plasma and brain had fallen to zero and RBC ChE was less than 50%.

Tremors, a slight diuresis in the last month, and a slight growth retardation was observed in the 0.03% rats. The plasma, RBC, and brain ChE were depressed.

The plasma, RBC, and brain ChE activity were depressed in the 0.01% rats while the plasma and RBC ChE activity were significantly depressed (only slight reduction in brain ChE) in the 0.003% rats. The 0.001% rats exhibited reductions only in the plasma and RBC ChE activity.

3. Conclusion

The ChE NEL for rats in this study is less than 0.001% Dursban in the diet (10 ppm). The systemic NEL is 0.01% (100 ppm).

B. Short-Term (Subacute) Dietary Administration - Rats (Hazelton Lab., Inc; 174-114) 11/15/68 - 11/21/68

1. Procedure

Groups of 10 Charles River albino rats/sex were fed diets containing 0, 0.3, 1.0, 3.0, or 10.0 mg/kg/day of Dursban (97.5% a.i.) (Phase I). The 0 and 0.3 mg/kg/day rats were fed their diets for 13 weeks. The remaining 3 groups were fed at this level for 4 weeks, rested for 3 weeks, and then fed at 0, 0.03, or 0.10 mg/kg/day, respectively, for 13 more weeks (Phase II).

The rats were observed daily for mortality. Weekly records were kept regarding individual body weights, food consumption, and the physical appearance and behavior of each rat.

Cholinesterase activity was measured by the Frawley modification of the Michel Delta pH method at the following intervals:

Dosage (mg/kg/day)	Phase I		Dosage (mg/kg/day)	Phase II	
	RBC & Plasma ChE (weeks)	Brain ChE (weeks)		RBC & Plasma ChE (weeks)	Brain ChE (weeks)
0	1,2,4,8,13	13	—	—	—
0.3	1,2,4,8,13	13	—	—	—
1.0	1,2,4,5,5	—	0	1,2,4,8,13	13
3.0	1,2,4,5,6	—	0.03	1,2,4,8,13	13
10.0	1,2,4,5,6	—	0.1	1,2,4,8,13	13

2. Results

Phase I - the 3.0 mg/kg rats appeared hunched during the first 2 weeks of the study while the 10 mg/kg rats were thin and hunched throughout the treatment period. Eight females in this group experienced tremors. The growth of the test rats in the 3 and 10 mg/kg groups was depressed during the first week of treatment. The RBC and plasma ChE activity were depressed in all 4 test groups. ChE activity levels for the 1, 3, 10 mg/kg groups were normal following compound withdrawal. No compound effect on ChE activity in the brain was evident at the 0.3 mg/kg test level.

Phase II - The 0.10 mg/kg rats exhibited lowered RBC ChE levels in both sexes and lowered plasma ChE levels in females. Brain ChE activity was not altered. Neither systemic nor ChE deviations were observed in the 0.03 mg/kg rats.

3. Conclusions

The ChE NEL for rats fed Dursban in this study was 0.03 mg/kg/day.

C. Six-Month Feeding Study in Rats (Albany Med. Coll.; March 18, 1971). 82%

1. Procedure

Groups of 20 Sprague-Dawley rats/sex were fed diets containing 0, 0.03, 0.15, or 0.75 mg/kg Dursban for 6 months. The rats were examined daily and general observations recorded. Body weights and food consumption were recorded weekly.

Five rats/sex/group were bled at 2, 4, and 6 months for hematology and clinical chemistry. The criteria investigated included RBC and WBC counts, hemoglobin, hematocrit, glucose, sodium, potassium, and SGT. Plasma and RBC ChE determinations were conducted on the control and 0.75 mg/kg groups at 3, 5, 7, and 16 weeks and on all four groups at 6 months. Brain ChE activity was measured when rats were killed.

Five rats/sex/group were killed after 3 months and the remainder after 6 months. Five rats/sex from the control and 0.75 mg/kg groups were examined grossly and microscopical for pathological alterations. Specimens of heart, trachea, lungs, esophagus, stomach, small and large intestine, liver, pancreas, kidney, urinary bladder, gonads, prostate, uterus, pituitary, thyroid, adrenal, spleen, thymus, lymph node, CNS, and eye were stained with H&E and examined. Liver and kidney were also stained by Oil red O.

2. Results

Sixteen rats were found dead during the experiment. There was no indication of a dose-response relationship. All 16 exhibited various forms of murine pneumonia upon necropsy.

The 0.75 mg/kg rats exhibited a lowering of RBC and plasma ChE activity. Brain ChE was unaltered. No other changes were noted in any group of rats.

3. Conclusion

A ChE MEL of 0.15 mg/kg/day of Dursban can be ascribed to this study with the qualification that the method of determination of ChE activity was never identified.

D. Six-Month Study of Dowco 179 in Rhesus Monkeys (Macaca mulatta) (Albany Med. Coll.; March 18, 1971). #286

1. Procedure

Rhesus monkeys were orally intubated daily with Dowco 179 for 6 months by the following protocol:

Group #	No. Animals	No./sex	Dosage (mg/kg)
1	4	2M,2F	0
2	3	2M,1F	0.08
3	3	2M,1F	0.40
4	4	2M,2F	2.00

General observations were made daily and individual body weights were recorded monthly. Clinical chemistry (Calcium, phosphorous, glucose, BUN, uric acid, cholesterol, protein, albumin, bilirubin, LDH, and SGOT) and hematology (REC, WBC, hemoglobin, and hematocrit) values were determined at 2, 4, and 6 months.

Plasma and RBC ChE levels were determined by the pH stat method on all animals at 0, 1, 3 and 5 weeks and at 2, 4, and 6 months. Plasma and REC ChE values were also determined on 4 monkeys killed at 3 months. Brain ChE levels were determined by the manometric method on all animals at sacrifice. Liver samples were taken from each animal at sacrifice (3 and 6 months) for the determination of biphenyl hydroxylase activity.

One monkey/sex was necropsied from groups 1, 3, and 4 at 6 months. The following tissues were examined microscopically (H&E stain):

heart	esophagus	pancreas	medulla oblongata
trachea	small intestine	brain	spinal cord
lungs	large intestine	cerebellum	spleen
stomach	gall bladder	kidneys	thymus
liver	urinary bladder	gonads	eye
uterus	pituitary	vagina	
thyroid	bone marrow	adrenal	

2. Results

The RBC ChE activity was depressed in the 0.4 and 2.0 mg/kg dose groups. The plasma ChE activity was inversely related to the dose level.

One monkey died during the course of the study but necropsy did not reveal any evidence of treatment-related pathology.

3. Conclusions

The ChE NEL for monkey orally dosed with Dursban is 0.03 mg/kg/day.

E. Results of 93-Day Dietary Feeding Studies of 0,0-Diethyl 0-3,5,6-Trichloro-2-Pyridyl Phosphorothioate in Beagle Hounds (DOW; January 15, 1964). 112-118

1. Procedure

Groups of beagle hounds (4/sex in control group and 2/sex/test level) were fed diets containing 0, 0.006, or 0.002% (0, 1.8 or 0.8 mg/kg/day) Dursban for 93 days. The 0.006% dogs were fed 0.2% for the first 5 days of the study while the 0.002% dogs were fed 0.06% for 16 days. The reductions in dose levels were necessary due to the development of gross cholinergic symptoms.

The 0.02(A)% (5.8 mg/kg/day) dogs were maintained for 45 days on this level and then placed on control feed for the remainder of the 93 days because of the development of gross cholinergic symptoms. The 0.02 (B)% (3.4 mg/kg/day) dogs were added to the study after 1 1/2 months, maintained on this level for 27 days, put on control feed for 5 days due to similar cholinergic symptoms, and returned to the test level for the last 2 weeks.

The dogs were weighed weekly and daily food consumption records were kept. Serum urea nitrogen, SGP, SGPT, and brain ChE determinations were made using the Technicon Auto-Analyzer. Plasma and RBC ChE activity was measured by a modification of the Michel electrometric method. Hematological values (hematocrit, hemoglobin, RBC, and total and differential WBC) were obtained on days 0 and 76 of the study.

All dogs were necropsied at the conclusion of the study. The lung, heart, liver, kidneys, spleen, brain, and testes were weighed. Portions of these organs, as well as spinal cord, peripheral nerve, pituitary, thyroid, parathyroid, adrenals, aorta, lymph node, thymus, esophagus, stomach, small intestine, large intestine, pancreas, gall bladder, urinary bladder, skeletal muscle, ovary, and uterus were microscopically examined (H&E stained).

2. Results

The 0.02% dogs exhibited extreme decreases in RBC and plasma ChE values. The brain ChE values of the 0.02(B)% dogs were decreased at necropsy. There was a marked decrease in the RBC and plasma ChE values of all the dogs in the 0.006% and 0.002% groups. The brain ChE of F dogs on these 2 doses also showed a great reduction.

A decrease in food consumption in the 0.02% dogs was noted and the body weights of the 0.02(B)% females were similarly reduced. The gross cholinergic symptoms seen on different occasions in the higher dosage levels include dilated and watery eyes, loose stools, vomiting, rough coats, labored breathing, and tremors of the legs and head.

3. Conclusion

The ChE NEL in dogs in this study was not determined. The ChE NEL for Dursban would be less than 0.002% of the diet or 0.08 mg/kg/day. *should read 0.6 mg/kg*

F. Blood Cholinesterase Activity in Dogs Receiving Diets Containing Dursban (Dow, April 21, 1964). 112118

Dosage (ppm)	No. Dogs	Duration (days)	Plasma ChE (% of control)	RBC ChE (% of control)
60	2	9	16	no effect
20	2	35	20	" "
6	2	35	50	" "
2	2	28	1 dog no effect 1 dog 50-81	" "
0.6	2	12	no effect	" "

ChE activity measurements were made according to the Technicon Auto-Analyzer procedure.

G. Oral Administration - Dogs (Eazelton Lab., Inc.; 174-115) 111313
112118

1. Procedure

Dursban (97.5% a.i.) was administered in gelatin capsules to 28 young adult beagles for 90 days using the following protocol:

Group No.	No. of Dogs		Level mg/kg/day	Interval
	M	F		
1	2	2	0	Entire study
2	2	2	0.03	" "
3	2	2	0.10	" "
4	2	2	0.30	" "
5	2	2	1.00	" "
6	2	2	1.00	Days 1-18
			0	Days 19-42
			0.03	Days 43-58
			0	Days 59-77
			0.10	Days 78-94
			0	Days 95-124
7	2	2	0.01	Days 1-32
			0	Days 33-45

Daily records were kept on general appearance, behavior, and pharmacotoxicological signs. Body weights and food consumption were recorded weekly. The dogs in Groups 1 thru 5 were tested for plasma and RBC ChE activity by the Technicon Auto-Analyzer using the Wright and Humiston method on Days 0, 2, 4, 8, and 16 and by the Frawley modification of the Michel Δ pH method on Days 16, 32, 64, and 90. The dogs in Group 6 had the determinations performed on Days 2, 4, 8, and 16 for dosages of 0.03 and 0.10 mg/kg after baseline values were obtained on the control diet. The dogs in Group 7 were tested on Days 0, 2, 4, 8, 16, and 32. The ChE determinations in Groups 6 and 7 were made using the Δ pH method.

All dogs were necropsied at the conclusion of the study. Brain weights were recorded. Brain ChE determinations were conducted.

2. Results

No ChE inhibition was noted in either the RBC or plasma by the Wright and Humiston method; hence, the Δ pH method was instituted. Dursban did not cause a significant lowering of the plasma or RBC ChE at the 0.01 mg/kg/day level. When administered at 0.03 mg/kg/day, plasma ChE was reduced but not RBC ChE. Dursban levels at 0.1 mg/kg/day produced a reduction in both plasma and RBC ChE activity but not in brain ChE. The effect of Dursban on ChE activity is reversible.

3. Conclusions

Based upon RBC ChE inhibition, the NEL of Dursban in dogs in this study is 0.03 mg/kg/day.

H. Safety Evaluation of Dowco 179 in Human Volunteers (Albany Med. Coll.; March 1972). 112 h5

1. Procedure

Sixteen healthy adult male volunteers received graded doses of Dowco 179 daily in a tablet form. The volunteers were divided into 4 groups and received 0 (49 days), 0.10 (9 days), 0.03 (22 days), or 0.014 (27 days) mg/kg/day.

Blood samples were tested twice a week for RBC and plasma ChE activity. The determinations were performed according to the procedures of Nabb and Whitfield and utilized a Sargent automatic recording pH stat in the titrametric measurements. Hematology (hemoglobin, hematocrit, and total and differential WBC) and serum chemistry (Ca, P, glucose, urea nitrogen, uric acid, cholesterol, protein, albumin, bilirubin, SAP, LDH, and SGOT) determinations were conducted at weekly intervals. Urinalyses (specific gravity, color, turbidity, pH, and determinations of cellular content) were also performed on a weekly basis.

2. Results

No treatment-related effects were noted by the urinalysis, hematology, or serum chemistry studies conducted.

RBC ChE activity was not inhibited at any of the treatment levels. Plasma ChE activity was depressed at the 0.1 mg/kg/day level and was slightly depressed (but not significantly) at 0.03 mg/kg/day.

Urine samples were examined for the presence of Dowco 179, its oxygen analog, and the decomposition product 3,5,6-trichloro-2-pyridinol with negative results.

3. Conclusions

Based upon RBC ChE inhibition, a Dowco 179 NEL for humans was not determined in this study. The NEL level must be stated as being equal to or greater than the highest level administered, 0.10 mg/kg/day.

I. The Effects of Dursban Insecticide on Blood Plasma Cholinesterase in Chickens (Dow; January 1969). 112118

1. Procedure

This study was subdivided into 3 test groups of varied treatment levels and test durations. Dursban was administered via the drinking water to 30 birds/treatment level. Group A treatment levels were 1 ppb, 1 ppm, 1 ppt, and non-medicated controls. Test duration for the 1 ppb, 1 ppm, and controls was 84 days. Body weights and blood samples were taken 7, 14, 21, 28, and 84 days after starting treatment. The 1 ppt level was discontinued after 1 week due to adverse effects on weight gain and ChE values. The 100 ppm level was initiated at this time and was designated as Group B (duration 77 days), sharing the controls of Group A. Group C treatment levels were 100 ppm and 0 and the duration was 2 weeks.

Plasma ChE values were determined by Biochemical Research Laboratory personnel with the Auto-Analyzer. Whole blood ChE values could not be determined due to clogging of the dialyzer by RBC.

2. Results

Dursban reduced plasma ChE values of the 100 ppm birds at all sampling periods. The data indicate no demonstrable effects at 1 ppb or 1 ppm. There also was no effect shown during a 2 week testing period of 10 ppm Dursban administered via the drinking water.

3. Conclusions

TB would prefer not to ascribe a NEL based upon plasma ChE inhibition. Since these were the only results obtained, we will cite only that plasma ChE inhibition was noted at the 100 ppm level.

J. Effect of Dursban in the Drinking Water of Chicks (J. Econ. Ent., 64 (5): 1179-1183, 1971).

1. Procedure

Three experiments were conducted to determine the effect of Dursban to white Leghorn cockerel chicks.

a. Experiment I - Dursban was offered in the drinking water to 3 replicates of 10 chicks/treatment level at 0, 0.32, 1.25, 5, 20, 80, 320, or 1280 ppm a.i. for 3 weeks.

b. Experiment II - To determine if the effects noted were due to the solvent and inert ingredients, xylene and Tween 80 (24.5 parts: 34.7 parts) were offered to the chicks at 152.9 and 651.8 ppm in the water. Tween 80 was offered by itself as 717.7 ppm in water. There were 2 replicates of 10 chicks/treatment and the duration was 3 weeks. The whole blood ChE activity was determined on 4 chicks/treatment level at the end of the experiment on a Spectronic 20 spectrophotometer.

c. Experiment III - Fifteen chicks/treatment were offered 0, 0.08, 0.32, 1.25, 5, 20, or 80 ppm Dursban in the water for 5 weeks. ChE activity was determined weekly on 3 chicks/treatment level.

2. Results

Most treatment related effects occurred at levels of 80 ppm or greater. Doses of 320 or 1280 ppm were highly toxic to the chicks and produced damage to the myelin sheaths of nerves in the white matter. Deaths occurred in the chicks given 80 ppm Dursban in the water through the first 18 days of the study. The feces of the 80 ppm chicks and several 20 ppm chicks were tacky and adhered to the down around the vent sealing it. The 80 ppm chicks had a depressed rate of growth and inhibition of the whole blood ChE was observed. The studies on the xylene and Tween 80 revealed no effects.

3. Conclusions

The ChE NEL in this study for Dursban is 20 ppm in chicks.

K. Chronic Toxicity of Dursban in Chickens, 1969 (J. Econ. Ent., 63 (1): 319, 1970).

Four week old unsexed White Leghorns were fed a diet containing 0, 25, 50, or 100 ppm Dursban for 4 weeks. Plasma ChE was determined by the electrometric method of Michel. The birds were weighed weekly and feed consumption was recorded daily.

Clinical Results

No.	Dose (ppm)	(lb feed/lb gain)	ChE (% of activity remaining at end of test)
5	0	3.46	92
5	25	3.65	78
5	50	3.72	73
5	100	3.76	60

XII. Chronic Toxicity

A. Results of Two-Year Dietary feeding Studies on Dowco 179 in Rats (Dow; September 20, 1971) 02118

1. Procedure

Groups of 25 Sherman strain 7 week old rats/sex/level were fed diets containing 0, 3.0, 1.0, 0.1, 0.03, and 0.01 mg/kg/day for 2 years. Supplementary groups were set up as follows to provide animals for interim pathological examination and periodic ChE determinations:

Group Assignment	No. rats/sex/level
1 week ChE	5
1 month ChE	5
3 month ChE	5
6 month ChE	5
9 month ChE	5
12 month ChE	6
12 month necropsy	5
12 month + 7-8 week recovery, necropsy, and ChE	7
18 month ChE	7
18 month necropsy	7
	57/sex/level

The rats were observed frequently for changes in appearance or demeanor as well as for mortality. Body weights were recorded twice weekly for the first month, weekly during months 2-6, and bi-weekly thereafter. Food consumption was recorded continuously during the first 3 months and one week/month thereafter.

Hematologic studies (PCV, hemoglobin, PBC, and total and differential WBC) were conducted on 5 rats/sex from the 0, 3.0. and 1.0 mg/kg/day groups at 1, 6, 12, 18, and 24 months. Urinalyses (total solids, pH, albumin, sugar, occult blood, and ketones) were conducted on 5 rats/sex from the same groups at the same time periods. Plasma and PBC ChE activity was determined from the supplementary animals included for this purpose. Brain ChE determinations were conducted at necropsy at 6, 12, and 18 months. After 2 years, blood and brain ChE were measured on all surviving M and all but 4 to 5 F/level. A modification of the pH-Stat method was used for all ChE determinations. BUN, SAP, and SGPT were measured at 12 and 18 months as well as at 2 years on all M survivors and the 4 to 5 F/level not used for ChE determination.

Necropsies were conducted on the rats included in the study for that purpose as well as those which survived the 2 year study. The brain, heart, liver, kidney, spleen, and testes were removed and weighed. Portions of these organs and the following organs or tissues were examined microscopically (H&E stain):

eye	pituitary gland	thyroid gland
trachea	parathyroid gland	esophagus
lungs	stomach	small intestine
aorta	pancreas	mesenteric lymph nodes
colon	urinary bladder	accessory sex glands
ovaries	skeletal muscle	sciatic nerve
uterus	spinal cord	adrenal gland
sternum	sternal bone marrow	
any nodules or masses suggestive of tumor development or other pathologic processes		

Histopathological examinations were conducted on the tissues of rats of the control, 3-0, and 1.0 mg/kg/day doses at 12 months and from rats of the control and 3.0 mg/kg/day groups at the other necropsies. Tissues of all rats with gross evidence of tumor development were also subjected to histopathologic evaluation.

2. Results

No alterations or deviations attributable to treatment were noted by any of the following criteria:

appearance and demeanor	mortality
body weight	food consumption
hematology	urinalyses
clinical chemistry	organ weight
gross pathology	histopathology

The plasma and RBC ChE activity was consistently reduced in the 3.0 and 1.0 mg/kg/day rats of both sexes. The brain ChE was inhibited in the 3.0 mg/kg/day rats. The RBC ChE of the 0.1 mg/kg/day F was inhibited at 2 of the 8 test periods (30 and 365 days). The remaining 6 determinations were normal.

3. Conclusion

Based upon RBC ChE activity, the NEL for rats fed Dursban for 2 years is 0.1 mg/kg/day. (2 ppm)

B. Results of Two-Year Dietary Feeding Studies on Dowco 179 in Beagle Dogs (Dow; December 10, 1971)

1. Procedure

Dowco 179 mixed with ground Purina chow was fed to Beagle Dogs for up to 2 years at levels of 0, 3.0, 1.0, 0.03, or 0.01 mg/kg/day. Groups of 3 dogs/sex/dosage level were fed for 1 year and necropsied immediately or after a 3 month recovery period in Phase A. Groups of 4 dogs/sex/dosage level were fed for 2 years in Phase B. All dogs were observed daily for changes in demeanor. Body weights were recorded weekly the first 6 months and bi-weekly thereafter. Food intake was measured weekly

during months 1-3 and 1 week/month thereafter.

Hematologic studies (PCV, Hgb, RBC, total and differential WBC, prothrombin) were conducted on all the 0, 3.0, and 1.0 mg/kg/day dogs twice pre-test and at 1, 3 (A), 6 (B), 12, and 24 (B) months. Urinalyses (specific gravity, pH, sugar, albumin, microscopic sediment exam) were performed on the same dogs at pre-test 1, 12, and 24 (B) months. BUN, SAP, SGOT, and SGPT were measured on all Phase A dogs twice pre-test and after 1, 3, 6, and 12 months. All Phase B dogs were tested for these compounds twice pre-test and after 1 and 24 months. The 0, 3.0, and 1.0 dogs were also sampled after 6, 12, and 18 months. BSP was measured on all Phase B dogs twice pre-test and terminally and on the 0, 3.0, and 1.0 dogs after 12 months.

ChE activity in the plasma and RBC of all dogs was determined 2(B) to 3(A) times prior to feeding the test diets, and after 1 week and 1, 3, 6, 9(A), 12, 15(B), 18(B), and 24(B) months. RBC and plasma ChE was measured on all dogs (A) placed on the recovery diet and RBC ChE was measured on the 0, 3.0, 1.0, and 0.1 dogs at 6 weeks and the 0, 3.0, and 1.0 dogs after 3 months on control feed. Brain ChE was measured on all dogs necropsied after 1 and 2 years and on the 0, 3.0, and 1.0 mg/kg/day dogs placed on recovery for 3 months. A modification of the pH Stat method was used for all ChE determinations.

The Phase B dogs were given complete physical examinations prior to termination including routine neurologic and ophthalmoscopic evaluations. Following gross necropsy examinations, the heart, liver, brain, kidneys, spleen, and testes were removed and weighed. Microscopic examinations were conducted on the following tissues from the 0, 3.0, and 1.0 mg/kg/day dogs from Phase A and from 0 and 3.0 Phase B dogs (H&E stain):

heart	pituitary gland	esophagus	sciatic nerve
liver	thyroid gland	lungs	spinal cord
brain	parathyroid gland	aorta	sternum
kidneys	small intestine	stomach	sternal bone
			marrow
spleen	mesenteric lymph nodes	pancreas	adrenal gland
testes	urinary bladder	colon	
eye	accessory sex glands	ovaries	
trachea	skeletal muscle	uterus	

2. Results

No treatment-related signs were noted by the following criteria:

Appearance and demeanor	Body weight
Food consumption	Hematology
Urinalyses	Clinical Chemistry
Ante-mortem physical examination (Phase B only)	
Gross and microscopic post-mortem examination	

The mean liver/body weight ratio in male dogs receiving 3.0 mg/kg/day Dowco 179 was increased. The plasma ChE was significantly depressed at dosages of 0.1 mg/kg/day ^{??} for 1 year in the Phase A dogs and 541 days in the Phase B dogs. The plasma ChE of the 3.0 and 1.0 mg/kg/day dogs was depressed throughout the study period. The 0.03 mg/kg/day dogs exhibited a depression in plasma ChE at some sampling times. Plasma ChE returned to normal within 2 weeks after being fed control diet. The ChE activities of RBC of dogs receiving 3.0 and 1.0 mg/kg/day were depressed. RBC ChE activity returned to pre-test levels in male and female dogs maintained on control feed for 3 months subsequent to receiving doses of 3.0 and 1.0 mg/kg/day Dowco 179 for 1 year. Brain ChE activity was slightly depressed in dogs receiving 3.0 mg/kg/day for 2 years.

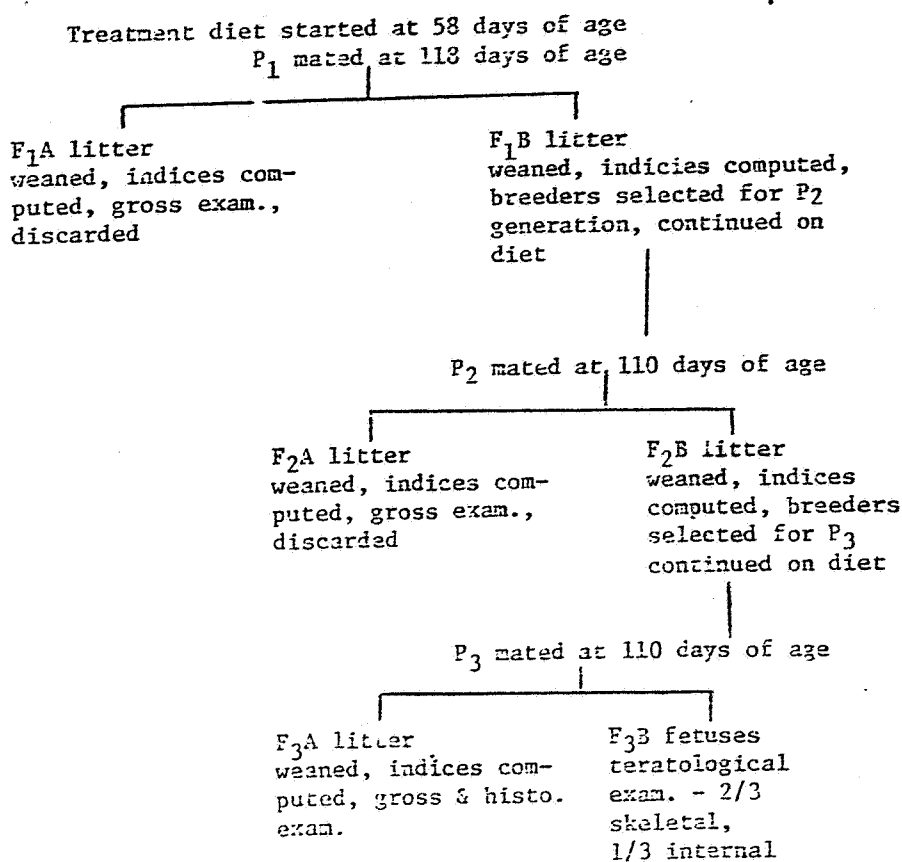
3. Conclusions

The ChE NEL in dogs fed Dowco 179 for 2 years based upon RBC and plasma ChE inhibition is 0.1 mg/kg/day. (4 ppm).

XXXX. Three Generation Reproduction and Teratology Study in the Rat
Following Prolonged Dietary Exposure to Dursban (Dow; August 20,
1971) 0240

A. Procedure

Groups of 10 M and 20 F Sprague-Dawley rats/dosage level were fed diets containing 0 (20M and 40 F), 0.03, 0.1, or 0.3 mg/kg/day of Dursban. Beginning with the P₂ generation, the rats were fed diets containing 0, 0.1, 0.3, and 1.0 mg/kg/day of Dursban. The study may be represented diagrammatically as follows:



For each mating, the number of copulatory plugs, number of conceptions, litter size, stillbirths, deaths, number of pups weaned,

and pup weights on the day of birth and at 5 and 21 days were recorded.

After weaning the F₃A litter, P₃ females were rebred to obtain F₃B fetuses which were used for teratological examination. These P₃ females were fed the test diet continuously except during organogenesis. During this period, the chemical was administered by gavage on days 6 through 15 of gestation. On day 20 the females were killed and the fetuses removed for cesarean section and examined for external abnormalities. Two-thirds of each litter were prepared for alizarin red staining and skeletal examination. The remaining one-third was prepared for soft tissue examination by the method of Wilson. Detailed examinations were conducted on the 1.0 mg/kg and control groups only.

Rats were observed frequently for clinical signs of adverse drug effect. Body weights and food consumption were recorded at weekly intervals. In the teratology study, body weights of females were obtained on days 0, 6, 15, and 20 of gestation and food consumption was measured for the gestational intervals 0-6, 6-16, and 16-20.

The following indices were computed from the reproductive data:

$$\text{Fertility index} = \frac{\text{No. of pregnancies}}{\text{No. of matings}} \times 100$$

$$\text{Gestation index} = \frac{\text{No. of live litters born}}{\text{No. of pregnancies}} \times 100$$

$$\text{Viability index} = \frac{\text{No. of rats alive at 5 days}}{\text{No. of rats born alive}} \times 100$$

$$\text{Lactation index} = \frac{\text{No. of rats alive at 21 days}}{\text{No. of rats alive at day 5}} \times 100$$

Blood samples were collected from 11 control and 5 or 6 treated dams/level at the time of cesarean section to determine RBC and plasma ChE activity. Similar determinations were made on 5 males/group.

Gross pathological examinations were conducted on any animals that died spontaneously during the test period as well as 5 pups/sex/dosage level from the F₁A, F₂A, and F₃A litters on post-partum day

21. Histopathological examination of tissues were conducted routinely on pups from the control and treatment levels of the F₃A generation:

eyes	pituitary gland	thyroid-parathyroid glands
brain	salivary gland	mesenteric lymph node
trachea	esophagus	testes-epididymis
thymus	stomach	abdominal skin
lungs	adrenal gland	spinal cord
heart	pancreas	skeletal muscle
liver	duodenum	sciatic nerve
spleen	ileum	ovary
kidney	bladder	uterus
colon	prostate	sternum
femur	cecum	

B. Results

No treatment-related changes were observed in the study (reproduction or teratology phase) by any of the criteria studied except the ChE determinations. There was a decrease in ChE activity in the 1.0 and 0.3 mg/kg females in both plasma and RBC as well as in the 1.0 mg/kg males.

C. Conclusions

The ChE NEL for the 3-generation rat reproduction study is 0.1 mg/kg Dursban based upon RBC ChE inhibition (with reservation since test method not named). Dursban is not teratogenic at the levels (up to 1.0 mg/kg) used in this study. The NE level for reproductive effects is greater than 1 mg/kg (the highest level fed).

XIV. A Neurotoxicity Study of Dursban in Laying Hens (Dow; June 29, 1966)

Dursban was given via gelatin capsule as a single oral dose to a total of 23 Leghorn laying hens at dosage rates of 40, 75, 100, or 150 mg/kg with no evidence of delayed ataxia or paralysis up to 27

days post treatment. Three hens were used per treatment level. PAM (50 mg/kg) or atropine (1/10 grain) were administered i.p. to half of the birds at each treatment level to reduce initial cholinergic reaction. Ruelene (1000 mg/kg) was administered to a positive control group and typical delayed ataxia-paralysis symptoms were noted. No adverse delayed ataxia-paralysis symptoms were noted in any of the birds treated with Dursban. No initial toxic reactions were noted in the birds treated with 40 mg/kg Dursban. No microscopic pathology was reported.

XV. Potentiation Studies

A. Potentiation Studies with Dursban in Combination with Ruelene and Malathion (Dow; April 22, 1964)

Groups of 5 male rats were given a single oral dose of Dursban, Ruelene, Malathion, Dursban + Ruelene (50:50), or Dursban + Malathion (50:50) at various dosage levels to determine the LD₅₀. The Dursban + Ruelene or Malathion were administered jointly as well as each pesticide separately at 4 hour intervals.

Dursban	LD ₅₀ 0.245 g/kg
Ruelene	LD ₅₀ 1.02 g/kg
Malathion	LD ₅₀ 1.37 g/kg

	Expected LD ₅₀	Joint	Found LD ₅₀ 4 hr. apart
Dursban + Malathion	0.420 g/kg	0.135 g/kg	0.158 g/kg
Dursban + Ruelene	0.398 g/kg	0.373 g/kg	0.482 g/kg

Dursban + Malathion resulted in an approximate 3-fold increase over the expected LD₅₀.

B. Potentiation Study on Dowco 179 and Vapona Insecticide (Dow; January 13, 1970)

Dowco 179, Vapona, and a 50/50 mixture of these materials were administered to groups of 5 starved Sherman albino rats/dosage

level in a single dose. The rats were observed for 2 weeks.

Dowco 179	LD ₅₀ 118 mg/kg
Vapona	LD ₅₀ 59 mg/kg
50/50 mixture	LD ₅₀ 135 mg/kg

There was no potentiation when Dowco 179 and Vapona were combined in a 50/50 mixture (anticipated LD₅₀ 79 mg/kg).

XVI. Chronic Dermal Studies

A. Comparison of Cholinesterase Depression in Humans and Rabbits Following Exposure to Chlorpyrifos (Dow; August 16, 1971)

1. Human and Rabbit Skin Exposures

Dursban 6 Insecticidal Concentrate was applied to the skin of the back and abdominal areas (and covered with occlusive gauze-adhesive tape patches) of human volunteers and 8 adult rabbits for 12 hour intervals at dosage levels of 50 mg/kg (1 application), 25 mg/kg (3), 10 mg/kg (4), or 5 mg/kg (20). No skin irritation or redness was produced. Plasma ChE depression was produced in humans at the 25 and 5 mg/kg dose levels. All 3 multiple dose levels produced a depression in plasma and RBC ChE activity (pH-Stat method).

2. Human and Rabbit Exposures to Ultra Low Volume Cold Aerosol Fog Containing Chlorpyrifos

Four adult male volunteers and 3 adult rabbits were positioned 25 feet from a fogger and exposed to ultra low volume cold aerosol fog for 5 minutes. No ChE depression resulted in the human subjects while the rabbits experienced a 30% depression in plasma ChE and 9.2% in RBC ChE.

B. Results of Human Skin Exposure to Dowco 179 (Dow; July 20, 1970)

Dursban 6 Insecticidal Concentrate was applied to the skin of the back and abdominal areas of 7 volunteers for intervals of 12 hours each. Single applications were made at dosage levels of 1.0, 1.5, 3.0, 5.0, or 7.5 mg/kg and multiple applications of 2 or 3 applications at 25 mg/kg, 4 at 10 mg/kg, or 20 at 5 mg/kg were made with

12 hour non-exposure periods between applications. The individual receiving 3 applications at 25 mg/kg experienced a depression in plasma ChE. Negative results were received from a lymphocyte tissue culture to determine whether morphological alterations had taken place in the chromosomes of the genetic material.

C. Clinical Toxicity of Dursban Insecticide in the Dog After Multiple Dipping (Dow; November 11, 1964)

Forty adult mongrel dogs and 10 pups were dipped in 0.125%, 0.025%, 0.05%, and 0.10% solutions of Dursban insecticide in water. Dippings were repeated at 15-30 day intervals. The greatest number of dips given were 6 times at 15 day intervals for adult dogs dipped in the 3 lowest dilutions or 4 times at 15 day intervals for adult dogs dipped in the 0.10% solution. The greatest number of dips the puppies received was 3 times at 15 day intervals in a 0.025% solution. No evidence of clinical toxicity was seen in any of the adult dogs or puppies dipped. Gestation and parturition were normal in all pregnant bitches. Dipping did not appear to adversely effect their puppies.

D. Physiologic and Pharmacologic Effects of Dursban Insecticide in the Dog After Multiple Dipping (Dow; August 25, 1964)

Three dogs were dosed i.v. with 5 mg/kg (two doses) of 1 - epinephrine, norepinephrine, or acetylcholine or 200 mg/kg (single doses) strychnine or d-tubocurarine 6-8 days following 3 dips at 15 day intervals with 0.05, 0.025, or 0.0125% Dursban. Responses to the drugs were found to be essentially in the normal range with no unusual responses attributable to the pretreatment (the dipping).

E. Physiologic and Pharmacologic Effects of Ethel* in the Dog After Multiple Dipping II (Dow; October 13, 1964)

A similar procedure was followed to that described above except that the dogs were dipped 6 times and the drugs were administered 2-4 days following the last dip. No untoward or unusual reactions were produced.

* O,0-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothiate [Dursban]

F. Toxicity Studies on Turkeys Confined on Soil Treated with Dursban (Dow; December 17, 1968)

Turkey pens were sprayed twice at weekly intervals with Dursban 25W at the rate of 4, 8, or 16 pounds/acre. No toxicity was seen following the first application, but one turkey died and 5 other were affected (incoordination) in the pen treated 2 times with 16 pounds active Dursban/acre. No toxic symptoms were seen in any other turkeys.

G. Toxicity Studies on Turkeys with Dursban Insecticide Applied as a Spray to Range Pens (Dow; September 9, 1969)

Dursban Insecticide was sprayed at the rate of 2 lb. and 4 lb. active ingredient to grass, weeds, and soil in turkey range pens. It was applied 2 times at 14 day intervals and the turkeys were necropsied 14 days after the second application. There was no evidence of toxicity to the turkeys in either treated pen and tissues from these turkeys showed no abnormalities.

XVII. Metabolism Studies

A. Final Report on Safety Evaluation and Metabolic Studies on Dowco 179 (Albany Med. Coll.; March 18, 1971)

1. Initial study with Dowco 179 - ^{14}C and TCP - ^{14}C in rats. Rats which had received 2.0 mg/kg/day of Dowco 179 for 10 days were injected ip with Dowco 179 - ^{14}C (10 μC /rat). At 1, 2, 8, and 24 hours after treatment 3 animals were killed. Extremely variable Dowco 179 determinations in the liver were obtained.
2. Second study with Dowco 179 - ^{14}C and TCP - ^{14}C in rats. Fifteen rats (not previously treated) were given concurrent ip injections of 10 μC Dowco 179 - ^{14}C and 2 mg/kg of unlabelled Dowco 179. Individual animals were killed at 1, 2, 4, 8, and 24 hours. The amount of Dowco 179 recovered from the livers was quite variable. The concentrations of Dowco 179 found in liver as related to time after administration indicated no orderly pattern in the metabolic turnover of the compound.
3. Urinary Excretion of Dowco 179 and its metabolic products in rats. Male and female albino rats were fed a diet containing a daily dosage level of 0.75 mg/kg Dowco 179. Urine samples were collected after 2 and 4 months. Unaltered Dowco 179 in the urine accounted for a very small fraction of the ingested daily dose. The oxygen analog (0,0-diethyl-0,3,5,6-trichloro-2-pyridylphosphate) accounted

for a much larger portion of the ingested material. No other metabolite was detected.

4. Urinary excretion of Dowco 179 and the metabolite 3,5,6-trichloro-2-pyridinol in monkeys

Urine samples were collected from Rhesus monkeys which were administered (via stomach tube) 0.08, 0.40, or 2.0 mg/kg Dowco 179 for 16 weeks. Neither Dowco 179 or its oxygen analog was detected in the urine of these animals. The 3,5,6-trichloro-2-pyridinol metabolite was detected in the urine of all animals.

B. Basic Studies on Dursban Insecticide (Down to Earth, 22: 3-7, 1966).

A single dose of 50 mg/kg in corn oil was administered via stomach tube to rats. Urine and feces samples were collected and animals were killed at various time intervals. The majority of the radioactivity was eliminated rapidly via the urine (88 - 90% of dose as detoxified compounds lacking ChE inhibiting activity) and feces. Dursban tended to accumulate in fatty tissue but was rapidly eliminated from kidney, muscle, and liver.

C. Investigations on Dursban Insecticide Metabolism of [^{36}Cl] 0,0-Diethyl 0-3,5,6-Trichloro-2-pyridyl Phosphorothioate in Rats (J. Agri. Food Chem, 15: 132-138, 1967)

Approximately 10 mg of [^{36}Cl] Dursban was administered to male Wistar rats as a single dose via stomach tube. The radioactivity was eliminated rapidly via the feces (10%) and urine (90%). The products excreted were [^{36}Cl] 3,5,6-trichloro-2-pyridyl phosphate (75 to 80%), [^{36}Cl] 3,5,6-trichloro-2-pyridinol (15 to 20%), with traces of 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate. Only the unchanged compound accumulates in the tissues (fat).

D. Absorption, Excretion and distribution of 0,0-Diethyl 0-3,5,6-Trichloro-2,6- C^{14} -2-pyridyl Phosphorothioate (C^{14} -Dowco 179) in Rats (Dow; April 23, 1971)

Ring labelled C^{14} -Dowco 179 was administered to 2 Sprague-Dawley male rats in a single dose. The routes of elimination were urine (63 - 70%), feces (14-15%), and respired air (0.15 - 0.39%). The urinary metabolites were 3,5,6-trichloro-2-pyridinol and the "origin material." Radioactivity in the respired air was 93-99% C^{14}O_2 . Tissue levels of radioactivity were low with the highest levels occurring in fat and intestine.

E. Comparative Metabolism of Insecticides. I. Preliminary Studies of Ring Labelled O,O-Diethyl O-3,5,6-Trichloro-2-Pyridyl Phosphorothioate Breakdown with Rat Liver Microsomes (Dow; March 17, 1970)

Dursban was a substrate for rat liver microsomal enzymes only when NADPH cofactor was in the incubation. 3,5,6-Trichloro-2-pyridinol was the only major metabolite and was formed at a rate sufficient enough to explain the in vivo rate of metabolites eliminated in rat urine.

F. Metabolic Studies with O,O-Diethyl O-(3,5,6-Trichloro-2-pyridyl) Phosphorothioate (Dursban) Insecticide in a Lactating Cow (J. Agr. Food Chem., 16; 45-47, 1968)

Dursban per se was absent from the milk and urine of a dairy cow fed 5 ppm for 4 days. A compound characterized by retention time as Dursban was found in the feces and represented 1.7% of the insecticide fed. Two metabolites were excreted in the urine which had retention times identical to the methyl esters of diethylthiophosphate and diethyl phosphate. They represented, respectively, 35.9 and 26.8% of the total insecticide fed.

G. Absorption, Excretion and Distribution of 3,5,6-Trichloro-2,6-Cl¹⁴-2-pyridinol in Rats (Dow; April 23, 1971)

3,5,6-Trichloro-2,6-Cl¹⁴-2-pyridinol (1.38 mg) was administered via stomach tube to rats. The material was mainly eliminated unchanged in the urine. About 0.5% of the administered dose was eliminated as CO₂. Tissue levels were low in all cases.

H. An Analytical Method for the Determination of 3,5,6-Trichloro-2-Pyridinol in Animal Tissues and the Metabolism of the Pyridinol in Rats (Dow; July 30, 1970)

Male Sherman rats were given a single dose of 1 mg of Cl¹⁴-3,5,6-trichloro-2-pyridinol. The compound was rapidly excreted via the urine. The highest concentration in the tissues was about 0.1 ppm in the liver, kidney, and blood.

I. Investigations on Dursban Insecticide. Uptake and Translocation of [³⁶Cl] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate and [¹⁴C] O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate by Beans and Corn (J. Agri. Food Chem., 15; 127-131, 1967)

Most of the radioactive compounds administered were hydrolyzed. The main products formed were ethyl-3,5,6-trichloro-2-pyridyl phosphate, and 3,5,6-trichloro-2-pyridinol.

J. Investigations on Dursban Insecticide. Metabolism of O,O-Diethyl O-3,5,6-Trichloro-2-pyridyl Phosphorothioate and 3,5,6-Trichloro-2-pyridinol in Plants (J. Agri. Food Chem., 15; 870-877, 1967)

Dursban is not absorbed into the plant, although it accumulates on the surface of the roots. Once it enters the plant it appears to be metabolized to form primary hydrolysis products where the P is still attached to the 3,5,6-trichloro-2-pyridinol. The pyridinol undergoes metabolism with the liberation of chloride and the formation of several decomposition products.

XIII. Metabolite Studies

A. Acute Oral Toxicity - 3,5,6-trichloro-2-pyridinol (TCP)

Rat (M&F)	LD ₅₀ 1000 - 3000 mg/kg
Rat (M)	LD ₅₀ 794 (709 - 889) mg/kg
(F)	LD ₅₀ 870 (785 - 1009) mg/kg
Mouse (M)	LD ₅₀ 380 (333 - 433) mg/kg
(F)	LD ₅₀ 415 (367 - 469) mg/kg
Dog	LD ₅₀ >1000 mg/kg (no deaths occurred)

Clinical signs in the rats and mice included flaccid paralysis, dyspnea, mild hypersalivation, and death. A rigor mortis like rigidity of the body occurred within seconds after death. Animals surviving were normal in appearance and activity at 24 hours. Only transient signs were observed in the dogs: emesis, ptialism, diarrhea, transient mydriasis, and a slightly depressed gag reflex.

B. Subacute Toxicity

1. Results of 90-Day Dietary Feeding Studies of 3,5,6-Trichloro-2-Pyridinol in Rats (Dow; July 9, 1964)

a. Procedure

Groups of 10 rats/sex/dosage level were fed diets consisting of 0, 1.0, 0.3, 0.1, 0.03, or 0.01% TCP for 90 days. The rats were weighed twice weekly for the first 4 weeks and once a week thereafter. Food consumption was recorded for the first week.

Terminal hematological values (hematocrit, Hgb, WBC, and differential WBC) were obtained from 5F rats from the 0, 1.0, and 0.3% groups. At necropsy the lungs, heart, liver, kidneys, spleen, brain, and testes were weighed. Portions

of these organs, as well as spinal cord, peripheral nerve, pituitary, thyroid, parathyroid, adrenal, aorta, lymph node, thymus, esophagus, stomach, small and large intestines, pancreas, urinary bladder, ovary, prostate, uterus, and skeletal muscle were examined microscopically (H&E stain). Bone marrow smears were prepared from the femurs of 5 rats/sex from the 0 and 1.0% groups and stained with Wright's Stain. Samples of blood were obtained for the determination of serum urea nitrogen content and alkaline phosphatase activity.

b. Results

Retardation of growth was found in both M and F 1.0% rats. Food consumption was decreased in the females and normal in the males. Both sexes showed evidence of diuresis during the entire 90 day period (also in 0.3% rats) and dry, bloody noses during the first month.

c. Conclusions

This metabolite (TCP) of Dursban has a systemic NEL of 0.3% in rats (150 mg/kg).

2. 91 Day Toxicology Study in Beagle Dogs Treated with 3,5,6-Trichloro-2-Pyridinol (Dow; August 26, 1970)

a. Procedure

Three beagle dogs/sex/dosage level (4/sex in control group) were fed diets containing 0, 1, 3, 10, or 30 mg/kg/day TCP for 91 days. Routine physical examinations (attitude, general body condition, temperature, pulse and respiratory rates, heart and lung auscultation, and direct and indirect ophthalmoscopic examinations) were conducted initially and terminally. Body weights were recorded weekly.

Routine hematologic (hematocrit, Hgb, RBC, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes, erythrocyte sedimentation rate, and total and differential WBC) and blood chemistry analyses (alkaline phosphatase, SGOT, SGPT, bilirubin, serum proteins, blood glucose, BUN, prothrombin, and partial thromboplastin times) were conducted on days -34, -18, 0, 14, 29, 59, and 91. SAP, SGPT, and SGOT were also determined on day 36 for dogs in the 10 and 30 mg/kg groups.

Urine analyses (color, turbidity, pH, specific gravity, protein, ketones, bilirubin, blood, glucose, and microscopic examination of urinary sediment) were conducted initially and terminally on all dogs and at 1 and 2 months on the 30 mg/kg dogs.

All dogs were grossly examined at necropsy and the brain, pituitary, liver, kidneys, gonads, spleen, heart, adrenals, and thyroids were weighed. The following tissues from control and 30 mg/kg dogs were examined histologically (H&E stain):

brain (fore-, mid-, hind-brain)	prostate
eyes	urinary bladder
adrenal glands	kidneys
pituitary gland	pancreas
spinal cord (2 levels)	stomach
salivary gland	liver
trachea	gall bladder
thyroid gland	intestine (5 levels)
esophagus	mesenteric lymph node
lung	spleen
heart	skeletal muscle
aorta	sciatic nerve
ovaries	sternum
uterus	epididymis
testes	mammary gland

Histologic sections of liver from 1, 3 and 10 mg/kg dogs were examined with oil RO, periodic acid Schiff (with and without diastase digestion), and Luna-Ishak method for bile canaliculi.

b. Results

Abnormal findings were confined to several of the blood chemistry tests conducted on the 30 mg/kg group. Elevated SGPT values were observed in all these dogs. Abnormally high SAP values were obtained for 1 dog in this group while 4 others had marginally increased values. The SGOT values in these dogs were also slightly increased. The liver weights of the 30 mg/kg females were also significantly smaller.

No gross or microscopic lesions were observed. Both control and treatment animals were parasitized with ascarids.

c. Conclusions

The systemic MEL for TCP in dogs in this study was 10 mg/kg (400 ppm).

3. Results of 93-Day Dietary Feeding Studies of 3,5,5-Trichloro-2-pyridinol in Beagle Hounds (Dow; October 27, 1970)

a. Procedure

Beagle hounds (2/sex/group) were fed diets containing 0, 0.2 (A)*, 0.2 (B), or 0.05% TCP for 93 days.

The dogs were weighed weekly and daily food consumption records were kept. Blood chemistry (serum urea nitrogen, SAP, and BSP) and hematological values (hematocrit, RBC, Hgb, total and differential WBC) were obtained at the beginning of the study and after 78 days.

The lungs, heart, liver, kidneys, spleen, brain, and testes from all dogs were weighed at necropsy. Portions of each organ, as well as spinal cord, peripheral nerve, pituitary, thyroid, parathyroid, adrenals, aorta, lymph node, thymus, esophagus, stomach, small intestine, large intestine, pancreas, gall bladder, urinary bladder, ovary, uterus, and skeletal muscle were examined microscopically (H&E stain). Bone marrow from the rib of each control and 0.2% (A) dog were examined after staining with Wright's stain.

b. Results

Increased SAP values were obtained from 2/4 0.2% (B), 3/4 0.2% (A), and 4/4 0.05% dogs. Growth depression was observed in the 0.2% (A) dogs as a result of refusal to eat at various times and in the 0.2% (B) females. Swelling and coarse granularity of parenchymal hepatic cells of 1 dog/sex in the 0.2% (B) group and all the 0.2% (A) dogs was observed.

c. Conclusions

The NEL for dogs in this study is 0.05% of the diet consisting of TCP (approximately 15 mg/kg/day) (500 ppm).

C. Three Week Study on Cataractogenicity of 3,5,5-Trichloro-2-pyridinol as Part of the Dietary Intake of Pekin Ducklings (Dow; HH-134)

1. Procedure

Groups of 10 (20 in control group) White Pekin ducklings, 5 days of age, were fed diets containing 0, 1, 3, 10, 30, 60,

* 0.6% for 9 days, 0.2% for 9 days, 0.4% for 13 days, 0.2% for duration of the study.

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100, 300, or 1000 ppm TCP for 21 days. The ducks were observed daily and their eyes were examined prior to the start of the test and 1 to 2 times weekly. Prothrombin times were determined for the control, 300, and 1000 ppm ducks at the end of the study. All ducks that died during the test interval were examined at gross necropsy.

2. Results

Abnormal findings were confined to the 1000 ppm ducks. These ducks had slightly depressed weight gains, did not preen themselves, and had a ruffled feather appearance. Three ducklings in this group had elevated prothrombin times. Opacification of the lens was not observed in any ducks in the study.

3. Conclusions

TCP is not cataractogenic for ducklings at concentrations up to 1000 ppm.

SUMMARY OF RESULTS

Acute oral toxicity	Rat (M)	LD ₅₀ 118-245 mg/kg
	Rat (F)	LD ₅₀ 22-137 mg/kg
	Guinea pig	LD ₅₀ 504 mg/kg
	Mouse	LD ₅₀ 62-162
Subacute toxicity		
90 days	Rat	ChE NEL 10 ppm
13 weeks	Rat	ChE NEL 0.03 mg/kg/day
6 months	Rat	ChE NEL 0.15 mg/kg/day
6 months	Monkey	ChE NEL 0.08 mg/kg/day
93 days	Dog	ChE NEL 0.8 mg/kg/day
90 days	Dog	ChE NEL 0.03 mg/kg/day
9-27 days	Man	ChE NEL >0.10 mg/kg/day (9 days)
3 weeks	Chick	ChE NEL 20 ppm
Chronic toxicity		
2 years	Rat	ChE NEL 0.1 mg/kg/day
2 years	Dog	ChE NEL 0.1 mg/kg/day
3-Generation reproduction	Rat	ChE NEL 0.1 mg/kg/day
		Reproductive NEL >1.0 mg/kg
Teratology	Rat	Negative @ 1.0 mg/kg
Neurotoxicity	Chicken	Negative @ 40 mg/kg

* ChE method not identified

Metabolite Studies (3,5,6-Trichloro-2-pyridinol)

Acute oral toxicity	Rat	LD ₅₀ 784 - between 1000 & 3000 mg/kg
	Mouse	LD ₅₀ 380 - 415 mg/kg
	Dog	LD ₅₀ >1000 mg/kg
30 days	Rat	NEL 0.30 (150 mg/kg/day)
31 days	Dog	NEL 10 mg/kg/day
90 days	Dog	NEL 0.050 (15 mg/kg/day)
Interactogenic	Chick	Negative @ 1000 ppm

DISCUSSION

Based upon the ChE NEL of 0.1 mg/kg/day (4 ppm) for dogs fed Dursban for 2 years, the ADI for man is 0.5 mg/day. The theoretical maximum daily intake from the proposed tolerances is:

R.A.C.	Tolerance	% Diet	% in Diet
Fat of cattle	1 ppm	0.25*	.1038
Meat & meat by-products of cattle	1 ppm	1.25**	.1187
Turkey fat, meat, etc.	0.2 ppm	0.49	.1014
			.1039

The proposed tolerances on peaches and field corn (grain, green forage, and fodder) are negligible residue tolerances. Additionally, field corn is a feed crop rather than a human food crop. Therefore, the ADI for Dursban supports the proposed tolerances.

The major metabolite in both plants and animals is 3,5,6-trichloro-2-pyridinol. This metabolite is less toxic than the parent compound and is not a cholinesterase inhibitor as is the parent compound. TB is not concerned further at the present time with this metabolite.

TI defers to CB regarding the necessity for a residue tolerance in milk.

* 50% of lard (0.51% of diet)

** 10% of beef & veal calculated as fat (4.9% of diet x 1.15)

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RECOMMENDATIONS

Toxicology Branch finds that the toxicity data adequately supports the proposed tolerances for 0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate, a cholinesterase inhibitor, and its metabolite, 3,5,6-trichloro-2-pyridinol. TB defers to Chemistry Branch regarding the necessity for a residue tolerance in milk.



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Toxicology Branch
Registration Divisioncc:
Chemistry Branch
Ecological Effects Branch
Division Reading File
Branch Reading File
Reg. No. 3F1306
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