DUPLICATE
TXR-2607

D STATES GOVERNMENT — DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
FOOD AND DRUG ADMINISTRATION

Memorandum

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002607

DATE January 9, 1968

Petitions Control Branch (S-13)

Mr. H. R. Gittes
Division of Toxicological Evaluation
Petitions Review Branch

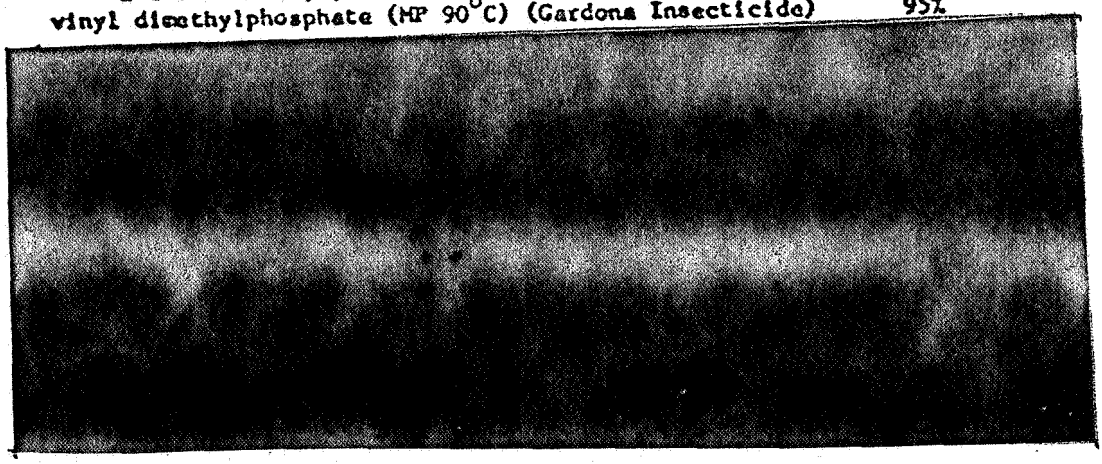
1. Evaluation of the toxicological data presented in support of petition for residue tolerances for Gardona.

PESTICIDE PETITION No. 8G0 665

Shell Chemical Company
New York, New York
(AF 3-553)

Components by weight of technical Gardona Insecticide are approximately as follows:

2-Chloro-1-(2,4,5-trichlorophenyl)--
vinyl diethylphosphate (MP 90°C) (Gardona Insecticide) 95%



Petitioner requests a temporary tolerance of 10 ppm for residues of Gardona Insecticide and its low melting isomer in or on apples. This is desired in connection with an experimental program to be conducted in areas throughout the U.S.

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TOXICOLOGICAL DATA

A. Chronic Studies Using Technical Gardona

1. Two-year feeding study - Rats

Note: The report included in the petition covers weeks 26-52 of a 2-year feeding study.

No. of animals used	Apparently 50M & 50F controls + 25M & 25F at each dosage level + a supplementary group for iterim sacrifice.
Method of administration	Diet
Diet levels	0, 5, 25, 125, 2000 ppm (0, 5, 25, 125 ppm for supplementary animals)

Results reported as follows:

No differences were seen between health and behavior of control and experimental rats.

Mortality and sacrifice during the 6 month period accounted for 5 male and 5 female rats. Autopsy showed lesions such as carcinoma and S. albus infection of the kidney. These were present in both control and treated animals and are considered spontaneous. Distribution of these rats among the dosage groups is not given.

Mean body weights of both sexes at the 2000 ppm level were reduced. At this level the females showed decreased food intake.

For the supplementary group (highest feeding level 125 ppm) sacrificed at 52 weeks health, behavior, body weight and food intake were similar to controls. Organ weights (brain, heart, liver, spleen, kidney, adrenal, thyroid and testes) were comparable to controls. Hemoglobin, hematocrit and erythrocyte and leucocyte counts were comparable to controls. BUN and serum protein were likewise unremarkable. Measurement of plasma, red cell and brain cholinesterase activity was negative except for an increase in the female brain cholinesterase activity at 125 ppm. This is probably incidental. Microscopic findings were not indicative of compound effects.

To date the "no-effect level" for this study is 125 ppm.

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2. Three-Generation Feeding Study - Rats

No. of animals used	120; 4 groups (20F & 10M per group).
Method of administration	Diet
Diet levels	0, 100, 330, 1000 ppm

Data was gathered as follows:

a. For the parents - observations on behavior and fertility and, at termination, body weights and gross autopsy.

b. For the offspring - litter size, survival weights at weaning. From the F_{3b} litters 10 males and 10 females of the control and high feeding levels as well as 5 males and 5 females from the intermediate levels were sacrificed. Body weights and brain, liver and kidney weights were recorded. Brain, heart, lung, liver, spleen, kidney, and testis were examined microscopically.

Results:

For the test parental groups appearance, behavior, body weights and gross autopsy findings did not differ significantly from the controls. Unexplained mortality was noted among the F_{2b} males fed the 1000 ppm diet, 4 out of the 12 selected for continuation dying on the same day and in the same cage. Tissues were autolyzed. No effects were noted upon fertility in terms of number or size of litters.

For the test offspring survival indices were comparable to controls except for the F_{1b} 330 and 1000 ppm weanling groups which had a higher survival rate then the controls. This is unquestionally due to the high mortality seen in some of the control litters. An increase in liver size was noted in the F_{3b} weanlings at the 1000 ppm level, especially for the males. No effects, however, were noted microscopically in the livers or any of the organs examined.

For this reproduction study a "no-effect level" of 330 ppm is established.

B. Subacute Studies

1. 90-day subacute feeding study - Rat

No. of animals used	210; 6 groups - 5 test, 15M and 15F per group; control 30M and 30F.
Method of administration	Diet
Diet levels	0, 12.6, 50, 200, 800, 3160 ppm.

Note: Compound loss from the diet was found to be 6-7% per week. Diet concentrations are therefore maximum and may have been reduced in the range of 18-28% during the 3 to 4 week diet preparation cycle.

Data was gathered as follows:

Appearance and behavior - daily
Food consumption - twice weekly
Animal weights - weekly
Whole blood cholinesterase at 4, 8, 12 weeks

Terminal studies:

For all animals--

Weights of brain, heart, liver, spleen, kidney, adrenal, thyroid, testis.

Gross examination of brain, pituitary, heart, liver, kidney, adrenal, small and large intestine, stomach, skin, skeletal muscle, salivary gland, lymph node, pancreas, eyes, ovaries, fallopian tubes, uterus, testes, prostate, urinary bladder, spleen, thyroid, parathyroid, lung.

For all feeding levels--

Hematology - Hb, PCV, RBC, WBC
Brain, red cell, plasma cholinesterase activity.
Blood chemistry - BUN, protein.

For the controls and high feeding level a differential leucocyte count as well as microscopic examination of the tissues listed for gross examination.

Results:

No effects attributable to the compound were seen in animal appearance, behavior, food consumption, blood chemistry. Autopsy and microscopic findings were also negative.

Compound related effects were noted as follows:

Increased liver and kidney weights, both sexes, at 3160 and 800 ppm. A suggestion of increased adrenal weight, males, at 3160 ppm.

Decreased cholinesterase activity for--

Whole blood at 4, 8, 12 weeks in both sexes at 3160 ppm.
Whole blood at 4, 8, 12 weeks in females at 800 ppm.
Red cell, at termination, in both sexes at 3160 ppm.
Plasma, at termination, in both sexes at 3160 and 800 ppm.
Brain, in females, at 3160 ppm.

Decreased hemoglobin and PCV for females at the 3160 and 800 ppm levels, possibly indicative of microcytic anemia.

A no effect level of 200 ppm based on both cholinesterase inhibition and systemic toxicity is established for this study.

2. 90-day subacute feeding study - Dog

No. of animals used	36; 5 groups - 4 test, 3M and 3F per group; control 6M and 6F.
Method of administration	Diet
Dietary levels	0, 50, 200, 800, 3200

Data was gathered as follow:

General health and behavior	- daily
Food intake	- daily
Weights	- weekly

Hematology (Hb, PCV, rbc, wbc, differential) at 0, 4, 13 weeks.

Cholinesterase	- Red cell and plasma at 0, 1, 2, 4, 8, 13 weeks. Brain at 13 weeks.

Urinalysis (pH, albumin, glucose, ketone bodies, microscopic) at 13 weeks.

Liver function test (BSP) at 7 and 13 weeks.

Terminal studies:

Organ weights - Brain, heart, pancreas, liver, spleen, kidney, adrenal, thyroid, testes.

Terminal studies: (cont'd)

Gross and microscopic examination of brain, spinal cord, thyroid, parathyroid, heart, lungs, spleen, liver, kidney, stomach, intestine, lymph nodes, bone marrow, pancreas, eyes, testes, prostate, ovary, Fallopian tubes, uterus, urinary bladder, skeletal muscle.

Results:

No effects attributable to the compound were seen in the urinalysis, BSP test, organ weights, gross autopsy and microscopic evaluation of tissues.

Compound related effects were noted as follows:

Hematology-hemoglobin and rbc count were significantly lower for males at the 3200 ppm level indicative of a normocytic anemia.

General health of the male dogs at 3200 ppm began to deteriorate at about week 7 and became progressively worse during the remainder of the study. This was marked by animals becoming thin and dehydrated and showing dull coats and hair loss.

Some indication of weight loss is seen in both sexes at the 3200 ppm level.

Decreased plasma cholinesterase activity for male dogs at 800 and 3200 ppm is seen beginning at week 1 and lasting throughout the study. For females similar effects are seen beginning in weeks 4 and 8 for the 3200 and 800 ppm diet levels respectively. Activity of red cell and brain cholinesterase is not affected although there is a suggestion of an effect upon the latter in the males at 3200 ppm.

"No effect levels" based on systemic toxicity is 800 ppm. For cholinesterase inhibition the "no effect level" is 200 ppm.

3. Subacute Dermal Study in Rabbits

Using 75% Wettable Powder

No. of animals	50; 3 groups - 2 test, 10M and 10F per group; control 5M and 5F.
Application	To clipped only and clipped + abraded skins, daily for 6 hours, 5 days/week for 3 weeks.
Dosage (as Gardona).	375, 1500 mg/kg

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Data was gathered as follows:

Physiological response
Body weight
Mortality

Terminal studies:

Organ weights of liver, kidney, heart, spleen.
Gross autopsy
Microscopic examination of liver, kidney, heart, spleen, lung, lymph node, gonad, adrenal, bone marrow, skin.

Results:

No compound related effects were seen in any of the parameters studied.

4. Two-week Subacute Feeding Study - Quail using Gardona

No. of birds	120; 4 groups (15M & 15F per group)
Method of administration	Diet
Feeding levels	0, 50, 200, 800 ppm

Data was collected on:

Mortality
Body weight
Food consumption
Egg production
Egg fertility
Egg hatchability
Chick mortality

Results:

No compound related effects were noted in any of the parameters studied.

C. Evaluation of Anticholinesterase Activity

1. Acute Percutaneous Effects - Rabbits - using 75% Wettable Powder

No. of animals	6; 2 groups (3M per group)

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1. Acute Percutaneous Effects - Rabbits - using 75% Wettable Powder (Cont'd)

Application	single 24 hour application to clipped skin
Dosage (as Gardona)	470, 1875 mg/kg

Whole blood cholinesterase activity determinations were performed for each rabbit 3 times prior to exposure and post-exposure at 2, 3, 7, 11, 14 and 21 days. Inhibition was determined in relation to the pre-treatment levels.

Results:

Depression in cholinesterase activity is noted at both dosage levels, effects at the lower dosage being somewhat marginal. At 470 mg/kg maximum depression (37%) was shown at 11 days. By 14 days recovery to 90% of initial activity had occurred. For the 1875 mg/kg dosage 47% reduction was observed on the 11th day with recovery to 78% of initial activity by 14 days. No data on results at 21 days is given.

2. Subacute Percutaneous Toxicity and Anticholinesterase Evaluation in Rabbits using 75% Wettable Powder and 2 lb/gal Emulsifiable Concentration

No. of animals	25; 5 groups (5M per group)
Application	6 hours/day, 5 days/week for 2 weeks to the clipped skin
Dosage levels (as Gardona)	100, 400 mg/kg for both preparations plus control.

Surviving animals were kept for 1 week following the exposure period.

Body weights are reported for days 5 and 12. Whole blood cholinesterase activity was determined for each animal 3 to 5 times prior to exposure and post-exposure at 3, 5, 10, 12 and 19 days.

Results:

Body weights and whole blood cholinesterase activities reported do not indicate any significant compound related effects.

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D. Study of Potentiation with other Anti-cholinesterase Compounds

Effect of 24 other chemicals upon the toxicity of Gardona was studied. Of the 24, eighteen were numbers of the class of cholinesterase inhibiting pesticides as listed in par. 120.3 1. (5) of the Pesticide Regulations. These were:

DDVP	Ethion	Phosdrin
Delnav	Folex	Ronnel
Diazinon	Guthion	Schradan
Dimethoate	Malathion	Sevin
Dipterex	Methyl parathion	Demeton
EPN	Parathion	Trithion

The remaining six were:

Bidrin	Co-Ral	Di-Syston
Ciodrin	Dibrom	Phosphamidon

The procedure was as follows:

a. The Acute Oral LD_{50} was determined for each of the compounds.

b. Each was then administered to a group of 10 rats at 1/2 LD_{50} to confirm the response expected (approx. 10% mortality).

c. Gardona was then administered at 1/2 LD_{50} in paired combination with each of the 24 compounds also given at 1/2 LD_{50}. Mixtures producing approximately 50% or less mortality were considered as being additive or less and were not tested further.

d. Mixtures showing more than 50% toxicity were evaluated by determining the LD_{50} of equitoxic mixtures and comparing the theoretical to observed LD_{50}.

Results:

Potentiation of the toxicity of Gardona was seen in combination with the following (increasing order as indicated by the theoretical/observed LD_{50}).

Co-Ral	1.17	*Dimethoate	2.13
Phosphamidon	1.37	*Parathion	2.29
*Sevix	1.37	*Guthion	2.33
Bidrin	1.55	*Ronnel	2.52
*Phosdrin	1.59	*Malathion	7.96
*Methyl Parathion	1.88		

*Note - listed in Pesticide Regulations.

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E. Acute Studies

1. Acute Oral Toxicity

Mice - LD_{50} > 5000 mg/kg

Rats - LD_{50} 4000-5000 mg/kg

In both cases Gardona was administered in 20% dimethyl acetamide + 80% 1,2-propanediol.

LD_{50} for male rats was found to be 2420 (2065-2840) mg/kg and for female rats 2295 (2065-2550) mg/kg when administered as a water suspension of the 75% wettable Powder. Toxicity in terms of Gardona would be 1815 and 1820 mg/kg for males and females respectively. Reactions of organo-phosphate poisoning i.e. salivation, lacrimation, diarrhea, tremors were noted.

LD_{50} for 2,4,5 trichloro mandelic acid was investigated, this being a possible metabolite of Gardona. For mice the LD_{50} was > 5,000 mg/kg, for rats the LD_{50} was in the range 500-1000 mg/kg. Both were dosed as 20% solutions in DMSO. No pre-death symptoms appeared in the rats which died. Surviving rats and mice were overtly asymptomatic.

2. Acute Dermal Toxicity - Rabbits

LD_{50} for Gardona and for the 75% wettable powder was > 2500 mg/kg, Gardona being applied as a 500 mg/cc solution in xylene, the wettable powder as a water suspension.

3. Rabbit Skin and Eye Irritation Tests

The 75% wettable powder is non-irritant to both the eye and skin of rabbits.

4. Acute Inhalation Toxicity to Rats

No compound related effects were seen in rats exposed for 1 hour to an atmosphere "saturated" with vapors of Gardona. Dried air at 4 l/min was first passed through a column containing 40 gm of Gardona then into the exposure chamber. Nominal concentration was not determined.

F. Demyelination Studies in Fowl using Gardona

No. of birds	24; 2 groups (12 per group)
Method of Administration	Capsule

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F. Demyelination Studies in Fowl using Gardona (Cont'd)

Dosage level a. 1.5 gm/kg (single dose)
b. 300 mg/kg daily for 5 days

For each dose all birds were premedicated with atropine sulfate 17.5 mg/kg and Protopam 50 mg/kg I.M. Microscopic examination was made for signs of demyelination of the anterior tibial nerves, sciatic nerves, spinal cord and medulla oblongata from those birds surviving at least 10 days.

Results:

Of the birds receiving a single dose, 4 died in less than 10 days. Of the group receiving multiple doses, 3 died prior to 10 days. No overt signs of demyelination were seen. Examination of the 17 birds surviving 10 days disclosed no histological evidence of demyelination, although a number showed microscopic evidence of neural lymphomatosis.

G. Animal Metabolism Studies

Study 1. Recovery studies in rats following injection of P^{32} labelled 2-chloro-1-(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate indicate that 85% of the phosphates recovered in urine was dimethyl phosphate. No intact original compound was recoverable.

Study 2. The metabolic fate of 2-chloro-1-(2,4,5-trichlorophenyl)-vinyl dimethyl phosphate was studied in rats and dogs using the C^{14} labelled compound, the C^{14} being placed at both vinyl carbons. Fate of the test compound was investigated by:

a. Recovery of C^{14} from urine, feces (for both species) and from expired gases, gut and contents, skin, hair and carcass for the rat.
b. Chromatographic separation and analysis from urine of metabolites, identification being made by co-chromatography.
c. Isotope dilution.
d. Enzymatic degradation with beta-glucuronidase.

Results:

For both species elimination appeared to be essentially via the urine and feces. In the rat, expired gases accounted for only 0.5% of the initial radioactivity. Recovery of major quantities of the radioactivity (Dog 90.5%; Rat 85.6%) were made within 48 hours. Analysis of the urine indicates that both species produce 2,4,5-trichlorophenylethandiol as the glucuronide, 2,4,5-trichloromandelic

acid, 2-chloro-1-(2,4,5-trichlorophenyl)vinyl methyl hydrogen phosphate. In the rat urine identification was also made of 1-(2,4,5-trichlorophenyl) ethanol as a uronic acid conjugate. No unchanged compound was found, which may indicate complete metabolism.

H. Wildlife Studies with Gardona

1. Toxicity to Fish

LD_{50} for coho salmon - 1.25 ppm (24 hr); 1.07 ppm (48 & 72 hr); 1.00 ppm (96 hr)

LD_{50} for rainbow trout - 0.81 ppm (24 hr); 0.68 ppm (48 hr); 0.38 ppm (96 hr)

LD_{50} for sunfish - 0.29 ppm (24 hr); 0.18 ppm (48 hr); 0.05 ppm (96 hr)

LD_{50} for spot - > 1.0 ppm

Mosquito fish - no mortality at concentration of approx. 0.05 ppm

Oysters - no effect at 1.0 ppm

LC_{50} for pink shrimp 0.42 ppm (24 hrs); 0.28 ppm (48 hrs);

For phytoplankton 1 ppm caused a 7.2% decrease in production.

2. Bee mortality - 5% when caged for 24 hours on foliage 1 day after treatment with 1.0 lb/acre. LC_{50} for bees was found to be 1.35 mcgm.

3. Toxicity to birds

Acute Oral LD_{50} for mallard ducks and chukar partridge - > 2000 mg/kg

Acute Oral LD_{50} for blackbirds 100 mg/kg

" " " " starlings > 100 mg/kg

Acute Oral LD_{50} to white leghorn cockerels 2528 mg/kg

I. Metabolic Studies in Plants

These were carried out in growing corn plants and apple seedlings using C^{14} labelled Gardona (labelling on both vinyl carbons). Investigation of the foliage for Gardona residues revealed the following relative amounts of Gardona and its metabolites in foliage of corn and apples.

	Corn	Apple
1. Gardona and its low melting isomer	80.0-84.7	46.9
2. Tetrachloro- and trichloroacetophenone	2.2- 3.3	1.6
3. 2,4,5-trichlorophenyl-α-(chloromethyl)-benzyl and 2,4,5-trichlorophenyl-α-methylbenzyl alcohols	1.0- 2.0	2.5
4. 2-chloro-1-(2,4,5-trichlorophenyl)vinyl methyl phosphoric acid	1.9- 2.2	4.2
5. Trichloro mandelic acid and/or trichlorobenzoic acid	2.0- 2.3	4.2
6. Unidentified polar materials	0.5- 1.3	2.0
7. Conjugate fraction	4.5-12.6	38.6

Subsequent study indicated that the conjugate fraction is composed principally of conjugates of metabolites 2 and 3 above in approximately equal proportions.

SUMMARY:

Two-year feeding studies in both dogs and rats are in progress at this time. Petitioner states that based upon verbal reports it is expected that for the dog the "no-effect level" will be 125 ppm. The data submitted in the petition on the 26-52 week period of the two-year rat study indicates decreased food intake and animal weights at the 2000 ppm diet level and "no effects" in the group sacrificed at 1 year and fed at a dietary level of 125 ppm.

In the 3-generation rat study an increase in liver size was seen in the F_{3b} weanlings of the 1000 ppm diet group.

Ninety-day dog and rat feeding studies disclose effects at diet levels of 800 ppm and above. In the rat liver and kidney sizes were increased, microcytic anemia may have been induced and plasma cholinesterase activity showed a reduction. At 3160 ppm rbc and brain cholinesterase

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activity was also reduced. For the dog, at 800 ppm plasma cholinesterase activity was reduced while at 3200 ppm, in the males, normocytic anemia became apparent, as did deterioration in general health. At that level too, both sexes showed weight loss.

Dosages of 1.5 gm/kg fed as a single dose and for 5 days at 300 mg/kg produced no evidence of demyelination in fowl surviving 10 or more days following administration of the test compound.

Cholinesterase inhibiting action was found to be potentiated when compared with 11 of the 24 compounds tested. With a single exception potentiation was of the order of 2.5 or less, the exception being malathion its magnitude being almost 8.

Data furnished with respect to plant metabolites, if found to be adequate do not indicate these to be factors mitigating against approval of the petition.

CONCLUSIONS:

1. Based upon 90-day studies in both dogs and rats the no effect dietary level is 200 ppm.

2. Based upon information obtained from incomplete 2-year feeding studies in progress in both dogs and rats the "no effect level" is 125 ppm.

3. In a 3 generation rat reproduction study the "no effect level" is 330 ppm.

4. The data are adequate to support the safety of the requested temporary tolerance.

INIT: HBlumenthal 1/24/68

cc: S-990
S-540
S-970 (Dr. Jacobson)
V-100
PP No. 8G0665

HRGittes:mmt 1/9/68
Revised: smr 1/23/68