10-11-78

DATE: OCT. 4, 1978

SUBJECT: Data Review for Chlorfenvinphos (Supona, Birlane, Dermaton Dip, GC 4072 SD 7859, etc). Requested by Burroughs-Wellcome and Co., RTP. Caswell #187.

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FROM: D.G. Van Ormer, Ph.D
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THRU: Reto Engler, Ph.D, Acting Deputy Chief
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10/11/78

THRU: Lamar B. Dale, Acting Chief
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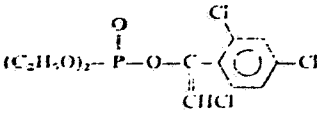
Description

Chlorfenvinphos, a vinyl phosphate insecticide has the chemical name 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate. The structural formula is given below :



Production batches of the technical material typically contain 89% trans- and 6.5% cis-isomers (beta and alpha isomers, respectively). The sum of the isomer weights constitutes a minimum of 92% of the total weight. The two isomers have similar oral toxicity in rats. Currently two formulations are registered by Burroughs-Wellcome: Dermaton Dip (Reg. No. 59-136) and Residual Surface Spray and Larvicide (Reg. No. 59-144). Intended uses include direct application to dogs, kennels, and food producing animals to control ectoparasites.

Summary

Both the recent additions to federal regulations for pesticide toxicology and the advances in concepts of toxicity testing have mandated revisions and additions to the toxicology data file for chlorfenvinphos. We present below a summary of recommendations to fill "data gaps" for chlorfenvinphos and also give the results of a recalculation of the MPI based on a lower NEL. (The revised MPI is not exceeded by the TMRC). Additional toxicological parameters are also suggested which, although not critical at this time for the chlorfenvinphos data file, merit further consideration in the regulatory field for organophosphates.

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A. Summary of Recommendations

(RPAR criteria triggered - mutagenicity, item 7)

1. Acute Inhalation Toxicity

We require for both the technical and formulated products an acute LC-50 determination with rats receiving "nose-only" or individualized exposure to a series of "analytical" concentrations. For formulated products attention should be given to obtaining values relevant to "use-conditions". (applies only to formulations representing inhalation exposure).

2. Primary Dermal Irritation and Sensitization

Determine Primary Irritation Index (according to Draize) with modifications necessary to compensate for the effects of systemic toxicity. Perform a dermal senitization study on formulations according to Buehler for formulations and uses representing a repeated exposure.

3. Primary Eye Irritation

Perform primary eye irritation study as described by Draize.

4. Subacute Dermal Study

Not present in file; submit study for those formulations and uses representing a repeated dermal exposure.

5. Potentiation

Labeling of containers for chlorfenvinphos and its formulations should specify that handling and applying the material should not be conducted together or in sequence with other organophosphates.

6. Neurotoxicity

We require a completion or repetition of the neurotoxicity study (Access. No. 090840, Vol 2, Part 1, Sec 23) to include histopathological examination of brain, spinal cord, and sciatic nerve tissue.

7. Mutagenicity

We ask OSPER, RD, OPP, to further consider the mutagenic potential of Supona. In view of the two positive mutagenicity tests, Tox Branch recommends oncogenicity testing in the rat and one other mammalian species.

8. Teratogenicity

Study not present. Perform study using two species; one should be the same species (rat) as used in the reproduction study.

9. Oncogenicity

We require oncogenicity testing in the rat and in one other mammalian species. Considerations should be given to the number of animals used and their viability.

B. Calculation of MPI

We feel upon review of current and past data that the NEL (RBC cholinesterase inhibition rat) should be set at 3.0 ppm rather than the previous 10 ppm. Also, we are re-adjusting the safety factor from 10 to 100, in view of hematologic abnormalities which appear at about the same dose level as the cholinesterase effect. Based on the readjusted NEL and safety factor the MPI (60 times ADI) calculates to 0.09 mg/day. This value is not exceeded by the TMRC (0.067 mg/day) a value based on current tolerances (40 CFR 180.322).

Whole milk consumption for infants (based on a 0.1-ppm Supona milkfat tolerance and 4% butterfat content) gives a TMRC of 0.004 mg/day. This value does not exceed the MPI of 0.012 mg/day for an 8-kg infant ingesting 1 kg of milk per day. For an adult on a milk-egg diet, the TMRC amounts to 0.0016 mg/day against an MPI of 0.09 mg/day.

C. Suggestions for Further Consideration

The proper relation of man to other species in regard to chlorfenvinphos toxicity requires further research. An approach toward this, specifically for chlorfenvinphos, has been suggested by P.J. Bunyan et al. Histochemically stained electrophoregrams of tissue extracts showed interspecies differences among birds in the inhibition of brain isoesterases. These enzyme characteristics correlated with interspecies differences in sensitivity to chlorfenvinphos. (Pest. Sci 2:148, 1971; see also P. Gennari et al., Pest Abst. 77-1073).

We recommend that attention should be directed toward the possibility of using the following toxicological parameters for assessing the consequences of environmental exposure to organophosphate pesticides: electroencephalogram (EEG) patterns (section L -6(a) and (d) this review) and electromyographic (EMG) measurements (section L-6(b) this review). These electrophysiological measurements have been reported able to detect adverse effects of organophosphates present at levels too low to inhibit cholinesterases.

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Review of Toxicity Testing

A. 1. Acute Oral Toxicity (Technical)
Medical College of Virginia, 1962

Four dosage groups of fasted rats, each group comprised of 10 males and 10 females, were gavaged at 5, 10, 15, and 20 mg/kg of chlorfenvinphos technical 1% (w/v) in propylene glycol. Most deaths occurred within a few hours of dosing.

Results

LD-50 (rats) = 10.9 mg/kg (females)
12.7 (males)

Toxic Signs: Usual cholinergic signs - fasciculation, salivation, lacrimation, and diarrhea. Post mortems were negative.

Tox Category: I.

Classification: Core - Guideline Data.

a. LD-50 estimated by chi-square normit method of Berkson.

b. Slope of dose-response curve not reported.

c. Post mortem organs should be specified.

d. Cholinesterase levels not reported.

2. Acute Oral Toxicity (Formulation)
Tunstall Lab., Shell Research, Ltd., London, 1968

Groups of 4 male and 4 female rats were used at unspecified dose levels. The animals were fasted, and the undiluted formulations of chlorfenvinphos were administered by gavage. Food and water were available for the 10-day observation period. The three 20% (w/v) formulations tested were described as follows:

[redacted] - based on [redacted]
EF 2462 - based on [redacted]
EF 2729 - based on [redacted]

Results

LD-50 (rats; based on formulation indicated) = 52.8 mg/kg [redacted]
34.4 EF 2462
77.4 EF 2729

Toxic signs: not reported.

Tox Category: I.

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INERT INGREDIENT INFORMATION IS NOT INCLUDED

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Classification: Core - Minimum Data.

a. Test substance not completely described.

b. Dose levels not specified.

c. Response data not tabulated.

d. Toxic signs and food consumption not reported.

e. Necropsy not reported.

Consideration of Inerts: (Memo, W.S. Cox 1972; Access No. 093395)

[redacted]

Toxicology status of Inerts induced from the following Tolerance Exemptions (CFR 180.1001):

a. On growing crops:

[redacted]

b. On either growing or post-harvest crops:

[redacted]

c. As pre-emergent solvent:

[redacted]

d. When applied to (food) animals: [redacted]

3. Other Acute Oral Studies

a) Determinations of rat oral LD-50 for chlorfenvinphos in various vehicles (peanut oil, dimethyl sulfoxide, polyethylene glycol) show ranges from 9.7 to 39.0 mg/kg. These studies are of supplementary value. In the following animals (for various vehicles) the oral LD-50 values are as follows:

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Mouse	117-200 mg/kg
Rabbit	300-1000
Guinea pig	125-250
Dog	5000-12,000

(EPA Access No. 090840, Vol 2, part 1.)

b) By injection of a 1% chlorfenvinphos emulsion in the tail vein, the i.v. LD-50 in the male albino rat is 6.6± 0.6 mg/kg. When injected into the cephalic vein of mongrel dogs as a 10% emulsion, the i.v. LD-50 is 50.4 ± 0.5 mg/kg (Shell Chem Co., 1963, Access No. 090840, Vol 2, item 15.)

c) A large species variation exists in acute oral response to chlorfenvinphos, with the rat the most sensitive animal tested. However, the above value of 5000-12,000 for the dog oral LD-50 appears anomalously high compared to the dog i.v. value of 50 mg/kg.

d) An albino-rat study on 21.1% EC chlorfenvinphos (Residual Surface Spray and Larvicide, EPA Reg. No. 59-144, Caswell No. 187, Review of D.G. Van Ormer, 26 Oct 77) gave an acute oral LD-50 of 44.6 mg/kg, based on total formulation. Five males and five females were used at each of six dose levels; and the animals showed typical cholinergic signs.

e) Evidence for low margin of safety, i.e. large slope of the LD-50 curve: a single oral dose of chlorfenvinphos (0.1 LD50) to rats had no effect on the following parameters: blood and brain acetylcholinesterase activity; serum cholesterol and glucose levels; adrenal ascorbic acid, adrenaline, and noradrenaline content.

(W. Tyburczyl, et al., Bromatol. Chem. Tyburczyk 7:465, 1974; CA 83, 001861G).

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Acute Oral Toxicities of SD 7859 (Access. No. 090840)

Species	Route	LD_{50} mg/kg (Approx)
Rat	(a) In propylene glycol 1% w/v solution	9.3 to 16.0
	(b) In peanut oil as 1% w/v solution	8.3 to 16.0
	(c) In peanut oil 1 ml/ 100g body wt	23 to 47
	(d) In DMSO 30% w/v solution	10 to 15
Mouse	(a) In peanut oil 1 ml/ 100 g body wt	131 to 173
	(b) In DMSO 3% w/v solution	150 to 200
	(c) -	117
Dog	(a) In propylene glycol or corn oil	>12,000
	(b) Undiluted	> 5,000
Rabbit	(a) In peanut oil	230 to 400
	(b) Undiluted	500 to 1,000
Fowl (White Leghorn breed) Chicks	(a) Undiluted	44 to 62.5
	(b) Polyethylene glycol/ water	36.6
Hens (2 yr)	(c) "	240 approx
Guinea pig	Aqueous suspension 10% w/v	125 to 250
Sheep	-	167.9
Calf	-	20
Cattle	-	MLD about 20

B. Acute Dermal Toxicity

1. Acute Dermal Toxicity (Technical)
(Medical College of Virginia, 1962)

Four dosage groups, each of 10 male albino rabbits, received undiluted compound 4072 to the clipped back area. The material was gently rubbed into the skin for three minutes with a glass rod. The dose levels were 0.15, 0.25, 0.35, and 0.50 ml/kg. Calculation was by the chi-square nomit method of Berkson.

Results

LD-50 = 417.0 mg/kg (95% conf. interval = 11.0%)
(rabbits)

Toxic Signs: Deaths occurred between the first (most deaths) and eight overnights after dosing. Early deaths were preceded by usual cholinergic signs - fasciculations, salivation, lacrimation, diarrhea. Postmortem findings were negative. No apparent damage to the skin.

Tox Category: II

Classification: Core - Minimum Data

a. Abraded animals not used.

b. Animals not individually housed.

c. Weights and food consumption not reported.

Additional Notes: A value of 31 mg/kg for the male rat acute dermal LD-50 has been reported by DHEW (Wayland Hayes, Jr., 1962).

The dermal LD-50 of undiluted test material applied to male albino rabbits was calculated as 412 $\pm$ 12 mg/kg. (Shell Chem. Co., 1963; Access. No. 090840, Vol 2, item 15).

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INERT INGREDIENT INFORMATION IS NOT INCLUDED

2. Acute Dermal Toxicity (Diluted Technical)
(Tunstall Lab., Shell Research, Ltd, 1967)

SD 7859 was applied as a 5% (w/v) solution [redacted] to the clipped necks of HL rats. Each dose level contained 5 male and 15 female rats. Dose levels were 50, 75, 113, and 169 mg/kg. After individual housing for 24 hours, surviviors were washed, and housed in groups for 10-days observation.

Results.

LD 50 = 91.8 mg/kg (95% conf-interval = 21%)
(rats; based on active component)
Toxic Signs: Extreme hypothermia, possibly due to an antipyretic effect on the hypothalamus.

TOX. Category: I

Classification: Core-Minimum Data.

a. Test substance not neet.

b. No animals abraded.

c. Skin washed post exposure.

d. Method of calculation not indicated.

e. No necropsy.

f. Use of rats can be justified as "most sensitive animal by oral route."

3. Acute Dermal Toxicity (Formulation)
(Tunstall Lab. Shell Research, Ltd., 1968)

Groups of 4 male and 4 female rats were dosed at unspecified levels. Formualtions (20%) were applied undiluted to the shorn dorso-lumbar region and covered by aluminum foil fixed with adhesive tape. During the over-night exposure period, food was with held, but water was available to the individually caged animals. After exposure the treated area was washed with weak detergent. Food and water were available during the 10-day observation.

Results

LD 50 (rats; based on total 20% formulation)= [redacted]
140 mg/kg → EF 2462
74 → EF 2729
186 →

Composition of the formulations is discussed in Sec. A.2., p.4, this review.

Toxic Signs: Not reported.

Tox Category: I

Classification: Core - Minimum Data

a. Test material not completely identified.

b. Dose levels not specified

c. Toxic signs, food consumption not reported.

d. Response data not tabulated.

e. Rabbits not used.

f. Necropsy not reported.

g. Method of calculation not mentioned.

4. Other Acute Dermal Studies

a. A 19% chlorfenvinphos emulsion used as a sheep-dip produced 56% whole blood cholinesterase depression in 6 hours, indicating rapid absorption of chlorfenvinphos through the skin of lambs. (W.R. Pickering, The Veterinary Record 77, 1240 (1965) EPA Access. No. 090840, Vol 2, part 1, item 5).

b. Cattle sprayed with 0.1 - 0.5% labeled GC 4072 showed maximum radioactive material in the blood 2 hours after treatment, indicating rapid absorption of the insecticide by the skin of cattle. (W.F. Chamberlain and D.E. Hopkins, USDA, Kerrville, TX; Access No. 090840, Vol 2, part 1, item 7).

c. Compound SD 7859 produced mild diarrhea in rabbits after dermal application at doses in a range which yielded an acute dermal LD-50 of 1250-2500 mg/kg. (G.W. Newell, SRI, 1961; Access No. 090840, Vol 2, part 1, item 6).

d. Additional supplementary dermal data for rabbits giving an LD-50 of 3200-4700 mg/kg is based on too few animals, which gave very variable responses. (R.A. Kehoe, Kettering Lab., 1963; Access No. 090840, Vol 2, part 1, item 10).

INERT INGREDIENT INFORMATION IS NOT INCLUDED

C. Acute Inhalation Toxicity (Technical and Formulation)

Exposure to the mist of GC 4072 at a nominal concentration of 2.0 mg/l resulted after 14 days in death of 8/10 for female rats and 4/10 for male mice. A formulation of 48% [redacted] at the same nominal product concentration, produced a 14-day rate of 1/10 in male mice (R.A. Kahoe, Kettering Lab., 1963; Access No.090840, Vol 2, Part 1, Item 10).

Tox Category: Not assigned; estimated at Category I for technical material.

Classification: Supplementary Data.

Recommendation: Due to the high oral toxicity of the test material additional data on rat inhalation toxicity are requested. We require an acute LC-50 determination performed with rats receiving "nose-only" or individualized exposure to the vaporized or aerosclized (0.5 - 3.0 microns) technical material. The "analytical" as well as the "nominal"concentration must be determined.

In regard to the formulated products (Dermaton Dip, Pesidual Spray and Larvicide) consideration must be given to obtaining data relevant to use conditions (vapor or aerosol concentration, particle size distribution, humidity range, etc.). An LC-50 must be determined on these formulated products if feasible. Analytical concentrations must be used for all calculations.

D. Primary Dermal Irritation and Dermal Senitization (Tunstall Lab., Shell Research Ltd., 1965. Access No. 090840, Vol 2, Part 1, item 13).

Undiluted SD 7859 rapidly produces on the skin of guinea pigs an erythematous condition which does not increase with repeated applications. Very dilute solutions (e.g. 0.5% in olive oil) induced a comparable erythema within a few days. The report states that SD 7859 may be a primary skin irritant and the possibility of prolonged contact (as from clothes impregnated for mothproofing) should be investigated.

No evidence of skin sensitization was observed after dermal application of 0.1% SD 7859 in light liquid paraffin.

Classification: Supplementary Data.

Recommendation: Perform Dermal Irritation study including determination of Primary Irritation Index (according to Draize). Since approximately half the animals will die (acute dermal LD-50 = 417 mg/kg) with application of two 500-mg doses as specified by Draize Test,* the study should utilize

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*Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, Assoc. of Food and Drug Officials of the United States, Topeka, Kansas 1959.

a larger number of animals, and should employ separate animals to study "abraded effects" rather than applying two 0.5-g samples to the same animal.

Perform Dermal Sensitization study according to the method of Buehler (E.V. Buehler, Arch. Dermatol. 91 (1965): 171-175).

E. Primary Eye Irritation (Laboratory not specified.)

Each of six adult male rabbits received 0.1 ml of compound 4072 (48% EC based on xylene) into the right eye, the other eye serving as control. Eyes were examined at 1,2,3,4, and 7 days.

Results

Toxic Signs: On the first day two of the rabbits developed conjunctivitis, which did not affect response to light. By day four all treated eyes appeared normal. No systemic toxicity.

Classification: Supplementary Data.

1. Not enough data to permit scoring.

2. Compound not adequately described.

3. Laboratory not indicated.

Recommendation: Perform Primary Eye Irritation study as described by Draize (J.H. Draize, The Appraisal of Chemicals in Foods, Drugs, and Cosmetics. Assoc. of Food and Drug Officials of the U.S., Austin, TX, 1959.)

F. Subacute Oral Dosing

1. Three-Month Rat Study (Medical College of Virginia, 1963; Access No. 090840, Vol 1, Part 2, item 25).

Six dosage groups of rats (10 male and 10 female each) received dietary concentrations of GC 4072 at 0,3,10,30,100, and 1000 ppm for 12 weeks. Baseline cholinesterase (ChE) values were determined weekly in 5 rats per sex in each group for 5 weeks prior to treatment.

During the 12 weeks of treatment, plasma and RBC ChE levels were measured every other week. Rats not receiving ChE monitoring were sacrificed after the 12th week, while the remaining animals were placed on control diet to measure return of ChE activity post-treatment.All rats were finally sacrificed. Body weights were recorded once a week, and food consumption was determined over 3-day periods during the 4th and 12th weeks on treatment. Histopathologic studies were performed on controls and on 2 females and 3 males of the 1000-ppm group, sacrificed after the 12-week dietary treatment. Tissues examined were those specified by the "Core-Minimum" Guidelines, for high-dose level, except for the following omissions:

spinal chord	salivary gland
eye	testes
thymus	prostate
trachea	sciatic nerve
esophagus	ovaries
mammary gland	uterus

Tissues from other dose levels were not examined.

Results

Rats of both sexes at 1000 ppm showed cholinergic signs - fasciculations, tremor, and bloody discharges about the nares and eyes. Behavioral studies on low-dose animals were not performed.

Growth was significantly depressed in both sexes on 1000 ppm dietary chlorfenvinphos, and in males on 100 ppm. The depressed growth in rats receiving 1000 ppm tended to remain depressed during the 4-week post-treatment withdrawal period. There was no diminution of daily food consumption at either the 4th or 12th week. Females at the higher doses tended to eat more than controls.

For plasma ChE, statistically significant depression appears at 10 ppm in both sexes. For RBC ChE the depression is generally significant for females at 10 ppm, but only borderline for males at that level. After the 4-week withdrawal period, return to baseline plasma ChE was generally complete for both sexes, while male RBC remained depressed at 100 and 1000 ppm. The study was possibly biased against demonstration of full anti-cholinesterase effect by the choice of the pretreatment group showing lowest ChE activity as the control group for the 12-week dosing.

(The cholinesterase data was spot-checked by this reviewer via individual t-test calculations).

Histologic findings were negative except for moderate testicular atrophy in one rat at 1000 ppm.

Body-weight-gain NEL = 30.0 ppm.

RBC ChE-depression NEL = 3.0 ppm

Classification: Supplementary Data.

a) No hematology, blood chemistry, urinalysis, nor gross necropsy.

b) Age of rats not reported.

c) Reasons for lack of necropsy of specific animals not stated.

Other desireable (but less critical) protocol components not present:

a) Behaivor not studied at sub-cholinesterase-inhiting dose levels.

b) Excess animals to compensate for "pre-terminal" sacrifice not employed.

2. Three-Month Rat Study (Medical College of Virginia, 1963; Access No. 090840, Vol. II, Part I, item 26).

Using littermate distribution, 35 young male and 35 young female albino rats (Wister strain) were placed for 3 months on each of the following dietary levels of GC 4072: 0, 1, and 3 ppm. The animals were observed for survival, body weight effects, food consumption, and cholinesterase inhibition. At the end of the study hematologic values were also recorded.

Results

The survivor rates for controls were $\frac{30}{35}$ for females and $\frac{25}{35}$ for males. Suvivor rates for treated animals either did not differ significantly from controls or were slightly better.

Average daily food consumption for controls fell from values at 4 weeks of 106g/kg/day (females) and 103 g/kg/day (males) to values at the end of 3 months of 69 (females) and 61 (males). At the end of 3 months the food consumption at both dosage groups was remarkably close to controls.

Weight gains for both dosage groups during the 3 months treatment were also almost identical to controls. After 6-weeks of treatment, animals at all dose-levels (including controls) had fallen about 18% behind normal weight for Wister albino rats (The UFAW Handbook on the Care and Management of Laboratory Animals, 1972). In addition, by this time unexplained deaths started appearing in both test animals and controls.

A spot-check by this reviewer (via t-test calculations) verifies that there is essentially no evidence of anti-cholinesterase activity at either 1 or 3 ppm in plasma or RBC. (A borderline depression appears in plasma of males at 3 ppm).

Examination of hematological data showed white-blood cell counts at 3-times normal values (Pathology of Laboratory Rats and Mice, Cotchin and Poe, 1967) except for female controls which were 4-times normal.

Classification: Inadequate Data.

Consideration of controls (low survivor rate, impaired weight gain, high WBC count) leads to the conclusion that their stressed condition destroys the cogency of the data as it stands by itself.

We feel that since the two-year rat study* (which does not show a quantitative NEL) shows no evidence for cumulative anti-cholinesterase behavior, the above two supplementary three-month rat studies may be conjoined to show a 3-ppm cholinesterase NEL.

* in item G-1

3. Eight-month Rat Study (S.Z. Lozowski, Bromatol. Chem. Toksykol. 9: 47-58, 1976; CA 84, 160349-50; Pol.)

Oral administration of chlorfenvinphos to rats for 8 months at 0.1 LD-50/day (approx 20 ppm) enhanced contractile reaction of isolated stomach muscles to exogenous acetylcholine, and decreased blood sugar by 27% without affecting serum free fatty acid. No effect at lower doses.

NEL = Not determined (less than 20.0 ppm).

Classification: Adequate as adjunct study.

4. Six-month Rat Study (L. Klukowska, Bromatol. Chem. Toksykol. 9:267, 1976. C.A. 85: 187451T).

   Six-month oral treatment of rats with chlorfenvinphos at 0.1 LD-50 dose (approx. 20.0 ppm) had no effect on hemoglobin level, RBC count, nor WBC counts. However, 40.0-ppm administration increased lymphocyte count approx. 20%.

   NEL: Not determined (less than 40.0 ppm).

   Classification: Acceptable as adjunct study.

5. Sixteen-Week Dog Study (Tunstall Laboratory, Shell Research Ltd, England, 1965; Access No. 090840, Vol II, Part I item 29.)

   Description: Chlorfenvinphos was fed to Beagle dogs at dietary concentrations of 0, 0.5, 1.0, and 3.0 ppm for 16 weeks. Animals per dietary level are tabulated below:

Dietary Level	No. of Animals	
	Male	Females
0	5	5
0.5	3	3
1.0	4	2
3.0	1	1

   The following observations were recorded: general health, body and organ weights, hematological parameters, BSP clearance, autopsy findings, and cholinesterase activities in plasma, RBC's and brain homogenate.

   Methods: Chlorfenvinphos (SD 7859) Batch No. FC 1339 (92% w/w active matter) was supplied by Shell. The nominal concentration of chlorfenvinphos in the diets was verified by chemical analysis periodically during use. Animals were kept in individual pens in the kennel. Periodic examination included alertness, appetite, temperature, pulse, auscultation of heart and thorax, and examination of conjunctivae and mucous membranes. During an 8-week pre-test observation, the animals were accustomed to the control diet while growth rates and health were observed to be normal. The animals were fed once daily and food consumptions recorded. Baseline values for hematology and cholinesterase were obtained. Autopsy at the end of six weeks was accompanied by determination of cholinesterase activity in a homogenate of 8 g brain tissue removed at the level of the optic chiasma. The following tissues were stained by routine techniques: brain, spinal chord, thyroid, parathyroid, heart, lungs, spleen, liver, kidney, stomach, small and large intestine, lymph nodes, bone marrow, pancreas, eyes, testicle, prostate, ovaries, fallopian tubes, uterus, bladder, and skeletal muscle.

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Results

The general health and weight of the animals was apparently not affected. In males, increased thyroid and decreased testes weights in the 1-ppm group are significant at a level of p=0.10 (90% confidence interval).* Hematology appears normal. BSP clearance in the 1-ppm group was studied at the 7th and 13th weeks. There appears to be a significant (p= 0.05) decrease in BSP clearance for males. This cannot be verified from the data alone however, as the measure of dispersion is not reported. Plasma and RBC cholinesterase activities were tested periodically and at the end of 16 weeks of treatment. Values did not differ from controls when considered at the level of P = 0.05 (one sided). Neither did brain cholinesterase activity (measured at autopsy) show any effect of treatment at the P=0.05 level. Autopsy findings are presented in detail.

Although both treated and control animals exhibited parasitic infestation, no effect of chlorfenvinphos treatment was reported. The report suggests a 1-ppm NEL for dogs, and that a higher NEL (3 ppm) could not be substantial with this study because only one dog per sex was used at that level.

NEL (Beagle dog) = 1.0 ppm

Classification: Core-Minimum Data.

a. Only one animal per sex at high dose level

b. Blood chemistries not performed.

* Only one of four males at 1 ppm was noted to have microscopically atrophic testes.

G. Chronic Feeding

1. Two-Year Rat Study (Medical College of Virginia; Access No. 090840, Vol 2, Part I, Sec 27; date and initial page missing).

Supona was fed to groups of 30 rats of each sex at dietary levels of 0, 10, 30, 100, and 300 ppm for up to 103 weeks. The report presents observations on growth and feed consumption throughout the exposure period. Hematological and urinary analyses were performed at three-month intervals. The protocol specified measurement of cholinesterase weekly for the first three months and thereafter at three-month intervals. From each dietary level two-to-four rats were sacrificed after three months in order to determine organ/ body weight ratios and any histological effects.

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The remaining animals were sacrificed at the end of exposure. Histopathological examination involved the following organs: heart, lung, liver, kidney, bladder, gastroenteric, spleen, skeletal muscle, bone marrow, skin, brain, pituitary, thyroid, pancreas, adrenal, and gonad.

Results

Survival: Significant decrease in survival at 300 ppm in males at 91 weeks, by chi-square test (p=0.01 level) performed by this reviewer. Significant decrease at a lower concentration is quite possible, however, as control survival was very poor - females 18% survived, males 48%.

Body Weight: Rats (especially females) evidenced a diminished weight gain at 100 and 300 ppm. Both sexes either did not gain or actually lost weight during the second year at all treatment levels including controls.

NEL = 30 ppm (Systemic effects).

Food Consumption: Unremarkable.

Hematology: (Two-year data for males not reported at the high dose level - 300 ppm). At the end of two years there is a significant decrease in hematocrit in females at all treatment levels. NEL < 10 ppm.

Two-year hemoglobin values were low, compared to controls, for females at all treatment levels. For males, periodic hemoglobin values were high at 300 ppm, while at two years the male hemoglobin values were high at both 30 and 300 ppm. NEL < 10 ppm.

Female WBC counts were twice control value at two years in all treatment levels. These were accompanied by the typical decrease in lymphocytes and monocytes seen in moderate human organophosphate toxicity. Values for males although variable, were significantly high at 100 ppm starting at 18 months.

NEL < 10 ppm.

Urinalysis: Reported as comparable to controls for reducing substances and protein (data not tabulated).

Cholinesterase Activity: At eleven determinations during the two-year study, females showed 8 instances of significant (p= less than 0.05) average depression in RBC CHE at the 10-ppm level. Males showed 3 instances of significant RBC CHE depression at this lowest test level. Hence the study does not quantitate a NEL cholinesterase level.

Organ-to-Body Weight Ratios: After two years, male liver ratios were significantly higher at 100 ppm, but the effect was not shown at 300 ppm. NEL = 30.0 ppm.

Histopathology: Negative with respect to controls (decision based on tabulation of data provided by descriptions).

Classification: Core - Minimum Data.

a. RBC counts not reported.

b. Blood chemistries not performed.

c. Histopathology and urinalysis data not in tabular form.

d. Historical control data not presented.

e. Controls did not maintain adequate viability.

f. Behavior not described (CNS tests. etc.).

g. Pre-neoplastic lesions not graded.

h. Measurement of precision not included with all mean values.

A quantitative NEL cannot be determined in this study. The study is, however, adequate to demonstrate that under the conditions chlorfenvinphos did not show an oncogenic potential.

2. Two Year Dog Study (Medical College of Virginia, 1965; Access. No. 090840, Vol 2, Part I, Sec 30.)

Protocol: Dosage groups of 4 Beagle dogs (2 male, 2 females) at 6 months of age were placed on dietary concentrations of GC-4072 (96% technical) at 0, 30, 200 and 1000 ppm for two years. The dogs had previously been immunized or treated for several canine maladies. Food consumption was measured daily and the dogs were weighed once a week. Prior to treatment, baseline values were obtained for uninalysis, hematology and cholinesterase parameters. Blood chemistries performed at the end of two years included BSP, SGOT, SAP and BUN.

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Organ weights were obtained at sacrifice for heart, spleen, kidney, liver, and testes. Histological exams were performed on heart, lung, liver, kidney, bladder, spleen, gastroenteric, bone, muscle, skin, brain, pituitary, thyroid, pancreas, adrenal, and gonad.

Results

Survival: All dogs survived the two years in apparent good health, except for one control sacrificed in moribund state at the 97th week.

Body Weight: Unremarkable over the two years.

Food Consumption: Unremarkable over the two years.

Urinalysis: All dogs comparable to control at all observation periods. Data not presented.

Hematology: Not remarkable at 8 observation periods during two years.

Cholinesterase Activity: Plasma levels were significantly depressed at all diet concentrations through the first 9 months, thereafter only dogs on 1000 ppm showed plasma ChE depression at 21 and 24 months. RBC cholinesterase was significantly depressed only at the 1000-ppm feeding level consistently through the first 12 weeks, and sporadically thereafter.

Organ Function Tests: Unremarkable at the two-year observation period.

Weight Data: Weight gain and organ-to-body weight ratios were unremarkable except for a significant rise in the male liver ratios and decrease in the testes ratio, both at 1000 ppm.

Histopathology: Of the 12 treated animals, one exhibited cholestasis in the liver (200 ppm) and one developed testicular atrophy (1000 ppm). Overall NEL (dog) < 30 ppm.

Classification: Core - Minimum Data.

a. RBC count not reported.

b. Complete blood chemistries not performed.

c. Historical Control data not presented.

d. EEG change, CNS tests not reported.

e. Standard errors not included with all mean values.

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H. Reproduction (Hine Laboratories, San Francisco, CA, 1967; Access No. 090840, Vol 2, Part 1, Sec 32.)

Stock solutions of GC 4072 in acetone were added to rat food to prepare diet concentrations of 1.0, 5.0, and 15.0 ppm chlorfenvinphos(technical). Diets were prepared every week and stored at room temperature. Stock solutions were maintained refrigerated. Choice of dose levels was based on a prior study which showed effects at a low dose of 30 ppm. Each diet group consisted of 10 male and 20 female Long-Evans rats maintained on food and water ad libitum. Males were housed in groups of five per wire cage, while females were housed individually in plastic cages. Weanlings, designated F_0, were mated after 79 days on the treatment diets when they were 100 days old. Pups from the first litters were discarded at weaning and the parents were mated again. Randomly selected pups, excluding runts, from the second litters were maintained on the diets after weaning and mated in turn when 100 days old. This protocol was followed for three generations. Litters greater than ten in number were reduced to ten on the fifth day.

Results

The appearance and behavior of all animals was indistinguishable from controls. Gross examination of parents at sacrifice revealed no abnormalities with the exception of an F_1b male at the 5-ppm level which developed an inability to maintain balance, stated as due to labyrinthitis. Mean weights of parents did not show significant tendencies except for the F_1b males, which exhibited decreased growth at all three dose levels. (The report claims the difference at the high-dose level is not significant by the Dunnett Test; whereas by two-sided t-test, performed by this reviewer, the significance is at the 0.05 level). Since subsequent generations showed no significant retarded growth the weight loss of the F_1b males was considered of no relevant meaning.

Average weights of the 21-day old F_1b pups at all three doses were lower than controls at the $p=0.05$ level in a two-sided t-test by reviewer. (The report states that a significant difference exists only at the 1.0-ppm level).

Litter production ranged from 85 to 100 percent over the three generations. The ratio of pregnancies to matings (Fertility Index) for the six breedings averaged 98.3% (controls), 98.3% (1.0 ppm), 93.3% (5.0 ppm), and 92.0% (15.0 ppm). The reviewer judges that these percentages do not show a dose effect.

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There were no significant differences from controls in the number of pups per litter surviving at the 1st, 5th, and 21st day in all three generations.

The ration of litters with live pups to number of pregnancies (Gestation Index) was 100 at all levels in each generation.

The Viability Index (ratio of pups alive at day five to the number of pups born) shows an 80-90% viability in the F_3 generation on the 1.0- ppm diet. Control viability was 93%, but there was no evidence of dose relatedness. The F_{1b} and F_{2a} litters also showed significantly increased mortality by day five at the 1.0-ppm level. Only three other low viability ratios occurred at higher doses (at day five).

By day 21 the Lactation Index (ratio of pups weaned at 21 days to pups alive at 5 days) showed six survival ratios differing significantly from controls; but variations were neither dose nor generation dependent. Controls did not maintain good surviability.

Autopsies were performed on ten male and ten female F_{3b} weanlings, each from both the control and 15.0-ppm groups; and on five males and five females, each from the 1.0- and 5.0-ppm groups. Liver-, kidney-, and brain-to-body weight ratios showed changes which were not significant nor dose related. The following organs were submitted to histological examination: brain, heart, lung, liver, spleen, kidney, and testis. All tissues were reported normal.

Toxic Effects: None reported as dose related.

NEL: 15.0 ppm (reproduction) ✓

Classification: Core - Minimum Data.

a. Highest dose level should have produced toxic effects.

b. Protocol not completely described.

c. Statistical methods neither referenced nor consistently performed, e.g., a chi-square test was actually performed as a t-test.

d. Explanation not provided for the significantly high mortality rates at day five in the 1.0-ppm groups, even though the effect is not dose dependent.

Other desirable protocol components not present:

a. Postnatal functional deficiency not specifically reported.

b. Increased normal anatomical variants not mentioned.

c. Fertility of males not reported.

d. Males should have been housed by same arrangement (individually) as females. All animals should have been caged in material which will not leach trace metals to the animals.

I. Metabolism

1. a) Overview (Access. No. 090840 Vol 2, Part II, Intro)

Supona is completely metabolized in rats and dogs. A single oral dose of 14C-vinyl labeled Supona is quantitatively eliminated in rats, and 94% of eliminated in dogs within 4 days. Neither species shows a sex difference in elimination, and rats show only a small biological variation in total excretion data. The major excretion route for both species is the urine (87-90%). Unchanged Supona is stated to be completely absent from the urine and carcass when elimination is complete. Urine metabolite patterns are in conformity with the finding that dog liver microsomes perform oxidative o-dealkylation of Supona at a much faster rate than rat liver microsomes.

In rats the brain uptake of labeled Supona at time of death was some 100 times greater than that of blood. While in dogs a dose of Supona which almost completely inhibited plasma cholinesterase, produced brain and blood concentrations of the same magnitude. In rats the brain uptake of C-14 in Supona is paralleled by depression of both cerebral and erythrocyte cholinesterase, whereas in dogs, both the enzymes are essentially unaffected.

The pronnounced difference in toxicity of Supona between rats (oral LD-50 = 10mg/kg) and dogs (oral LD-50 = greater than 5000 mg/kg) is stated as due partially to the greater hepatic detoxification in dogs; but, especially to the much greater brain uptake of Supona by the rat, along with the greater sensitivity of brain cholinesterase in the rat.

Administration of an oral dose of labeled Supona (12.5mg) to a human male volunteer produced the same metabolites as in the rat and dog. Within 24 hours 94% of the dose was excreted in the urine.

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b) Additional Factors Cited for Large Difference in Interspecies Toxicity. (D.E. Hathway and D.H. Hutson, Biochem Pharmacol.16:949, 1967)

Dogs have a 15-fold greater tissue absorption-metabolism ability for Supona than do rats.

Rat RBC's showed a 3-fold greater uptake of Supona from rat blood than did dog RBC's from dog blood, a fact claimed as showing more labile binding of Supona in rat blood. Gel filtration, however, did not show a difference in degree of Supona binding by the two plasmas. In vitro measurement of rat-brain AChE sensitivity to Supona shows almost a 10-fold greater sensitivity compared to dog-brain AChE in homogenate.

Donninger,C., et al; (Tunstall Lab) Biochem. J. 126:701, 1972, CA 76: 122629B.

Large species differences were observed between rat, mouse, rabbit and dog in liver microsomal dealkylation of chlorfenvinphos. In liver slices the relative rates of dealkylation are 1,8,24, and 88, respectively.

2. Absorption and Elimination of GC 4072 Applied Dermally to Cattle. (Access No. 090840, Vol 2, Part II, Sec 6.)

Dermal spraying of cattle with 0.1, 0.25, and 0.5% chlorfenvinphos (EC) was conducted in a manner to result in "field condition" applications (approx. 2 liters). Absorption of the labeled compound by the blood of the cattle was a maximum at 2 hours, and by one week had decreased to about 43% of the maximum at 0.1% application, and about 11% of the maximum at the higher spray concentrations. Within one week, 25-32% of the applied dose was excreted in the urine. The amount found in the feces after one week was only 2% of the applied dose.

After i.m. administration of chlorfenvinphos (233 mg) to a cow, over a 5-day period, 0.2% of the dose appeared in the milk (fat 83%, whey 13%, insoluble protein 4%). Chlorfenvinphos concentration dropped from 0.08 ppm on day one to 0.0005 ppm on day five.

3. Consideration of Metabolites

a) Four of the five proposed Supona metabolites which do not show in rats and dogs a strong possibility for conjugation, are listed as having oral LD-50 (rat) values above 1000 mg/kg (Access No. 090840 Vol 2, Part II, Intro.) The fifth metabolite, 2,4-dichlorobenzoic acid, appears in Registry of Toxic Effects of Chemical Substances (NIOSH, 1976) with a subcutaneous LD-50 (mouse) of 1200 mg/kg.

b) Suspected Carcinogens, NIOSH, 1975.
None of the five "nonconjugated" metabolites is listed.

4. Consideration of Intermediates

a) Acute Toxicities and Skin Irritant Properties of Four Intermediates in the Production of Chlorfenvinphos. (Access No. 090840, Vol 2, Part II, Sec. 3).

The dermal LD-50's (rat) of the following four intermediates are all greater than 1500 mg/kg, with the exception of [redacted] which has an LD-50 of 600 mg/kg in female rats.

[redacted]

Prolonged skin contact induces dermatitis. Splashing into the eye causes conjunctivitis; permanent eye damage not expected.

[redacted]

Severe skin and eye irritant; even short exposure must be avoided. Systemic effects after dermal exposure appear worse than when ingested.

[redacted]

Typical organic-solvent effect on skin.

[redacted]

Skin and eye irritant; avoid contact. Permanent damage to eyes not expected.

b) Suspected Carcinogens, NIOSH, 1975

None of the above four intermediates is listed.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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c) P.J. Hanna and K.F. Dyer, Mutat. Res. 28(3): 405, 1975.

Spot tests with eleven strains of S. typhimurium (Ames) and with E. Coli (Bridges) were negative for triethylphosphate. However, recessive lethal mutations were produced in D. melanogaster at a rate of 69% accumulated mutations, compared to 9% control. Significant at the 0.001 level.

d) Memo, W.S. Cox, PE Br. PT Div., EPA, 8 April 1971.

There is no likelihood of formation of chlorodibenzodioxins either in synthesis or degradation of chlorfenvinphos.

Metabolism Comments:

1. Metabolites are stated to be conjugatable and/or excretable. Metabolism pathway represents detoxification. Bioaccumulation is not indicated. Only indirect evidence has been presented to relate man to other species in regard to Supona toxicity.

2. Further research is suggested to aid in assigning position to humans in the interspecies sensitivity scale for Supona: "Detection of Differential Inhibition of Brain Isoesterases by Electrophoresis, "P.J.Bunyan et al., Pesticide Sci: 2:148, 1971.

J. Potentiation

(Kettering Laboratory, Univ. of Cincinnati 1963; Access No. 090840, Vol 2, Part 1, Sec 24).

Weanling female rats (CD strain, 21-days of age) were housed 10 per dosage cage for a 3-week adaptation period. Each binary mixture was given orally as a freshly prepared suspension in peanut oil having a volume of 10 ml/kg body weight. The LD-01 of each compound was selected as the unit dosage for administration. A factorial design was used to evaluate each binary combination of 4072 and, consecutively, each of 22 other cholinesterase-inhibiting insecticides.

Results

No deaths occurred in any group given singly the LD-01 of the respective compounds, and none of the animals given only peanut oil died. Toxicity was sharply potentiated in each of the combinations of Compound 4072 with Diazinon, malathion, methyl parathion, and ronnel. For these mixes the mortality was 10/10 (9/10 for methyl parathion) among rats given an LD-01 dose each of 4072 and the specified compound. A milder potentiation was revealed for combinations of 4072 with Co-ral, Guthion, and parathion; and slight potentiation with Ciodrin, Dibrom, and Systox.

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The study also demonstrates that with regard to either the number of compounds potentiated or the degree of potentiation exhibited, compound 4072 is equalled or exceeded in potentiating ability by 7 other organophosphates.

G. Muacevic, Toxicol. Appl. Pharmacol 25:180, 1973.

Chlorfenvinphos potentiates the acute toxicity of bromophos-ethyl in the rat.

B. Wysocka - Parus, et al; Bromatol. Chem. Toksykol. 7:195, 1974; CA 81 164300.

A potentiation effect was observed in oral administration to rats of mixtures of chlorfenvinphos and either IPO 62 or IPO 63.

Recommendation

Labeling for containers of Compound 4072 and its formulations should specify that handling and applying the technical or formulated product should not be conducted together or in sequence with other organophosphates, unless positive knowledge exists for a lack of mutual potentiation for the mixtures involved.

K. Neurotoxicity

1. Medical College of Virginia, 1963; Acces. No. 090840, Vol 2, Part 1, Sec. 23.

White Leghorn hens, 16 months of age and weighing 1.5-1.9 kilos each were given ten daily intraperitoneal doses of GC-4072 (with atropine if necessary) dissolved in ethanol/propylene glycol. Dose levels were 0, 100, 150, 200, and 300 mg/kg. The number of hens used at each dose varied from one to six. Over half (13/22) of the birds died; the remainder were sacrificed 20 days after the tenth dose. Segments of brain, spinal chord, and sciatic nerve were preserved for possible future study. After each injection the hens showed intoxication at all treatment levels. During the 20-day post-treatment observation, no signs of intoxication or ataxia presented.

Classification: Supplementary Data.

1. Histopathology not performed.

2. Twenty-one days of observation required.

3. Ten surviving hens are required.

4. Dosing should be either by dietary administration or by oral intubation.

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2. Other Neurotoxicity Studies

(Cooper Technical Bureau, 1964; Access. No. 090840, Vol 2, Part 1, Sect. 20).

Intraperitoneal dosing of five adult atropinized hens (Rhode Island Reds) with LD-50quantity (300 mg/kg) of the commercial product (90% w/w chlorfenvinphos) showed no evidence of ataxia after 35 days. Another group of five similar, atropinized birds was dosed by the oral route on four occasions with 300 mg/kg over a period of 14 days (total dose 1200 mg/kg). Ataxia did not appear 35 days after the last dose, but 2/5 of the birds died due to acute toxicity.

Classification: Supplementary Data.

1. Histopathology not performed.

2. Insufficient number of birds.

3. Intubation or dietary dosing preferred to intraperitoneal administration.

Recommendation: We require a completion or repetition of the neurotoxicity study Access No. 090840, Vol 2, Part 1, Sec 23 to include histopathological examination of brain, spinal chord, and sciatic nerve tissue.

We note that the related organophosphate, diethyl-2,2'-dichlorovinyl phosphate is neurotoxic in hens at a sc dose of 18 mg/kg (EPA-600/1-76-025, July 1976, p. 79). In addition it gives 75% inhibition of neurotoxic esterase (NTE). The structure of chlorfenvinphos is similar to compounds inhibiting NTE, a property correlating very well with neurotoxicity.

L. Cholinesterase and Other Clinical Studies

The outstanding problem concerning Supona is the very wide reported species variation in acute toxicity and the difficulty in extrapolating experimental data to the assessment of effects in man. Studies on pages 29-33 are intended to increase the entree to risk assessment in the areas of dermal and worker exposure, dietary intake, and "subclinical" CNS effects. Incubation of GC-4072 with human plasma and red blood cells produced direct inhibition of ChE activity. Most sensitive was the plasma. A concentration of 1×10^{-8} M GC-4072 produced 50% inhibition of plasma ChE which was comparable to that produced by an equimolar concentration of paraoxon. On red blood cells 50% inhibition was obtained with 4.9×10^{-7} M GC-4072 as compared to 1.6×10^{-8} for paraoxon (Ambrose et al., Toxicol. and Appl. Pharmacol. 17:323, 1970).

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1. Acute Dermal

a) C.G. Hunter, Ind. Med. 38:50, 1969; Access. No. 090840, Vol 2, Sec 41.

Although the values are variable, human blood Supona concentrations, 24 hours after dermal application of 24% EC Supona, correlate roughly with plasma cholinesterase inhibition. For an estimated 0.20 mg/cm2/hr application, the plasma inhibition is 76% and whole blood Supona concentration is 14.6 ug per liter. RBC cholinesterase data, however, show unpredictable responses: 9% erythrocyte inhibition resulted 24 hours after absorption of 0.08 mg/cm^2/hr; however, other subjects receiving higher doses did not exhibit RBC ChE depression. The report states, nevertheless, that the findings suggest that single dermal exposures to 5 and 10 mg active ingredient per kg (in 24% EC formulation) for periods of 3-4 hours may inhibit RBC ChE. Thus, a dermal exposure (5 mg/kg, 24% EC) which reduced plasma and RBC cholinesterases by 45 and 20 percent, showed that plasma ChE had not returned to pre-exposure values by the 8th day.

b) Access No. 090840.

There is no clear-cut relationship between calf-blood cholinesterase inhibition and concentration of spray formulation i[redacted] Recovery of whole-blood cholinesterase (depressed 56% by dipping calves in 0.2% chlorfenvinphos) required 48 hours. Maximum inhibition occurred between 6 and 18 hours, indicating rapid adsorption through the skin. In a series of spray treatments on calves there is a tendency for the chlorfenvinphos effect to be more pronounced during the earlier stages of the treatment. This tendency to develope refractoriness has not been investigated for other species.

c) Tunstall Laboratory, Shell, 1969; Access No. 090840 Vol 2, Sec. 42.

In one case, approx. half of a 20 mg/kg dose (slurry of 25% wettable powder, Supona) applied to 1/20 of the body (forearms and hands of the human volunteer) was calculated as absorbed, and resulted in 82% depression of plasma cholinesterase and 13% depression of RBC cholinesterase. A period of 10-30 days is required for return of plasma cholinesterase to pre-exposure values. Toxic symptoms were not indicated.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

2. Worker Exposure (Wayland J. Hayes, Jr., pers. comm., Access No. 090840, Vol 2, Sec 9, 22 June 62). "Experienced workers applying GC-4072 have shown marked depletion of plasma cholinesterase (levels of about 0.15-0.28 as compared to normal pre-exposure values of about 0.8). RBC cholinesterase was not affected. Supona should be used with care".

3. Dermal Subacute

Shell Research, 1965; Access No. 090840, Vol 2, Part I, Sec. 13.

There was stated to be complete absence of "side effects" in guinea pigs even when plasma ChE was severely depressed(to 10% normal) by 100 mg/kg/day. The NEL for dermal exposure to SD 7859 for 15 days to Guinea pigs is less than 0.1 mg/kg/day.

4. Dietary Subacute

a. J. Barna and G. Simon, Cereal Res. Commun. 1973 , 1(3), 33 (Eng). CA 86:847g.

Birlane (10 and 30 ppm) administered in diets to rats did not significantly alter the body weight gain, food consumption, nor intestinal methionine and calcium absorption. The intestinal absorption of glucose was slightly increased, whereas that of sodium was slightly decreased. However, cholinesterase activity of blood and intestinal mucosa was markedly inhibited.

NEL (dietary, rats) = less than 10 ppm.

b. J. Latuszynska, et al., Bromatol. Chem. Toksykol. 1973, 6(3), 359 (Pol.) C.A. 80, 104642d

Parenchymal degeneration, hyperemia, and edema were observed in the heart, lung, liver, kidney, and spleen of rats dying from oral treatment with chlorfenvinphos (0.01, 0.02, and 0.1 of LD50/day; approx. 2.0, 4.0 and 20.0 ppm) for up to 3 months. NEL not determinable from this abstract due to insufficient information.

c. Tunstall Lab., Shell, 1967; Access No. 090840, Vol 2, Part 3, Sec. 7.

Daily dosing of pigeons with 2-8 mg/kg/day reduces the oral LD-50 of chlorfenvinphos from the single-dose value of 12 mg/kg to a 7-day repeated-dose value of 3 mg/kg.

A "self-potentiating" effect has also been observed with neurotoxic sign in hens, e.g., with long-term administration, the NEL is lower than with a single, equivalent oral dose (leptophos). Pesticide Induced Delayed Neurotoxicity, EPA-600/1-76-025 (1976), p.258-59.

d. P.J. Bunyan et. al., Pesticide Sci. 2:148, 1971; Pest. Abstract 72-0113.

Chlorfenvinphos is particularly toxic to pigeons (acute oral) compared to pheasant and quail. After dietary 100 ppm for 2-4 weeks, cholinesterase measurements were performed by conventional and electrophoretic measurements on extracts of liver, kidney and brain. Histochemically stained electrophoregrams offered a possible explanation of the interspecies toxicity difference as due to differential inhibition of brain isoesterases in the pigeon but not in the pheasant or the quail.

5. Physiological and Pharmacological Effects.

Tunstall Laboratory, 1965; Access No. 090840, Vol 2, Part I, Sec. 43.

In vivo studies

a. A rise in blood pressure in the rat accompanying i.v. administration of 2.5 times the LD-50 was considered somewhat anomalous for an anticholinesterase agent. Experiments with adrenegic blocking agents, adrenalectomized rats, and rats with severed vagi and spinal chords all indicated that intoxication is caused by discharges of the peripheral sympathetic nerve supply orginating in the central nervous system above the spinal chord. The report states that "Possibly (chlorfenvinphos) is active directly on the vasomotor area of the brain. . . due to lipid solubility. The hypertensive activity appeared to be acetylcholine dependent because it was reversed by intravenous atropine".

b. EEG recordings of the rat after a 5-times LD-50 i.v. dose of Supona showed activation and desynchronization. At 20-30 minutes after injection, high-voltage waves (10C/S) and wave complexes were observed.

c. Intravenous administration of chlorfenvinphos (50-150 mg/kg) to the rabbit produced effects similar to those in the rat, but with an additional squirting of blood and mucous from the lungs through the nose and mouth. Pre-medication with atropine or antihistamines did not prevent the pulmonary hemorrhage which also appeared to interfere with EEG measurement.

In Vitro Studies

a. Inhibition of Tissue Response to Acetylcholine or to Electrical Stimulation.

Supona at a bath concentration of 10^{-4} to 10^{-3} g/ml inhibited the response of frog rectus abdominis muscle to exogenous acetylcholine and of chick biventer cervicus muscle to electrical stimulation. At lower concentrations there was no effect. Phosdrin at 10^{-6} to 10^{-3} g/ml, on the other hand, potentiates these stimulations.

b. Inhibition of Intestinal Contractions.

Both rabbit and guinea pig small intestine showed increases (above normal) in contractile response at low concentrations of Supona when the tissue was washed after removal from baths containing Supona at 10^{-6} to 10^{-5} g/ml. (While in the bath the tissues were refractory to spontaneous contractions.)

Classification: Adequate as adjunct study.

1. Too few animals.

2. Dose levels not environmentally representative: approx. 70-fold higher than expected for dermal occupational blood absorption.

6. Other Electrophysiological Studies

a. Adverse Effects of Environmental Chemicals and Psychotropic Drugs, Vol I, 1975, 125-40, I. Desi. CA83:158599 A.

For rats on dietary 10-ppm Birlane the desynchronization-time (EGG pattern) became longer from the 5th day of dosing. A three-months feeding of 3-ppm Birlane did not produce these alterations. Rats on 3-ppm Birlane, however, showed increased wave amplitude after 6-7 days exposure to flashing light of 5 or 11 hz. (Such deviation points to an enhanced irritability of the CNS). Although rats on 1 ppm did not show the alteration to flashing light (throughout 43 days of examination), their spontaneous vertical orientation performance was reduced to about 50% of controls over a period of 7 days, before gradually rising to about 75% normal by the 37th day. Rats on 3 and 10 ppm showed slightly greater loss of orientation ability. The minimal dose giving depression of PBC and brain ChE was 10 ppm.

NEL = less than 1.0 ppm (orientation, performances, rats). (approx. 0.1 ppm by extrapolation).

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b. K.W. Jager et al., Brit. J. Ind. Med. 27:273, 1970.

"Seventeen workers out of 36 exposed to dimethylvinylphosphates showed electromyographic signs of impaired nerve and muscle function, while the blood cholinesterases did not show definite changes, possibly indicating that electromyographic detection is more sensitive." F. Matsumura, "Toxicology of Insecticides," Plenum, 1975.

c. I.L. Natoff, Eur. J. Toxicol. 3:363, 1970; Pest. Absts. 72-1301.

Among several anticholinergics injected intracerebrally (bypassing the blood-brain barrier) chlorfenvinphos was one of only two which were not several orders more toxic compared to other routes of administration. This fact supports the rapid blood-brain barrier permeability of chlorfenvinphos in vivo.

d. F.H. Duffy, J.L. Burchfiel, and V.M. Sim,"Persistent Effects of Organophosphate Exposure As Evidenced by Electroencepnalographic Measurements," Pesticide Induced Delayed Neurotoxicity, Proceedings of a Conference, p102, USEPA, Health Effects Research Lab., RTP, 1976. Available from Nat. Tech. Info. Svc., Springfield, Va. 22161. EPA-600/1-76-025.

Monkeys (2.5-4.0 kg) receiving 1 ug/kg of Sarin once a week for ten weeks (subclinical dose) showed a persistent relative increase in occipital-temporal (O-T) beta activity (in the EEG) similar to that seen in animals receiving a single larger clinical dose of 5 ug/kg. The increased O-T beta activity persisted for at least one year, at which time there appeared frontal-central (F-C) beta activity, which did not occur in the single dose group. Tissue cholinesterase in monkeys returns to normal within three months of exposure. Humans with documented exposures to O-P compounds also exhibited altered EEG patterns long after exposure and return of plasma and RBC cholinesterase to normal values.

The pathogensis of the long-term effects, whether due to anticholinesterase properties or to some direct lesion of axons or myelin, probably represents an unexpected and possibly irreversible extension of the primary drug effect.

Comments:

a. No "inhalation cholinesterase" values applying to field conditions were found in the literature.

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b. It appears that a significant component of the toxicity of chlorfenvinphos is due to central effects. The hypertensive activity is in common with Sarin, eserine and TEPP.

M. Mutagenicity

1. B.J. Dean (Tunstall Laboratory) Arch. Toxikol 30:67, 1972.

Chlorfenvinphos (E- isomer, one microdrop) failed to induce reversions in Escherichia coli WP2 when applied to a plate containing 10^8 bacteria per surface. Such reverse mutation systems in which perhaps mutation at a single locus is required to restore the original phenotype, are more specific than forward mutation, in which any number of loci may be involved. Selectivity of this nature makes a combination of test organisms necessary.

2. Burroughs -Wellcome Co., RTP, NC, 28 Mar 77; Access No. 232123, App 2.

Mutagenicity of Supona at the TK Locus in Mouse Lymphoma Cells.

When supplemented with rat liver metabolic activation, Supona at 90 ug/ml has about half the mutagenic potential (on a concentration basis) as 2-AAF, a moderate carcinogen. This mutagenic potential, however, was exhibited at a cell survival of only 1.5%.

3. Litton Bionetics, Inc., Kensington, M.D., Feb 1977; Access No 232123, App. 3.

Supona was tested for mutagenic potential in the standard Ames Salmonella assay (5 strains), Saccharomyces assay and in the E. Coli genetic-repair assay of Rosenkrantz. All assays were performed both in the presence and absence of in vitro metabolic activation. The results of the Salmonella and Saccharomyces assays were negative. A positive response in the E. coli repair assay occurred at the four highest doses of the repair-deficient strain. This effect was not dose-depehdent. Since no response was observed in the "repair-capable" strain, the report concludes that Supona could be damaging DNA in a manner capable of being repaired. This effect is generally regarded as indicating mutagenic potential.

4. Mutagenicity of Triethylphosphate (metabolized Supona intermediate). P.J. Hanna and K.F. Dyer, Mutat. Res. 28(3): 405, 1975.

Spot tests with eleven strains of S. typhimurium (Ames) and with E. Coli (Bridges) were negative for triethylphosphate. However, recessive lethal mutations were produced in D. melanogaster at a rate of 69% accumulated mutations, compared to 9% control. Significant at the 0.001 level.

Comments

1. Positive results for mutagenicity of Supona have been obtained in the following tests:

   a. TK locus in mouse lymphoma cells.

   b. E. coli DNA repair assay.

2. Since Supona gives two positive tests for mutagenicity, we ask OSPER, RD, OPP to further consider the mutagenic potential of Supona.

3. Tox. Br. recommends oncogenicity testing in the rat and one other mammalian species.

N. Teratogenicity

Study not present in Supona file. Perform study using two species; one species should be the same species (rat) as used in the reproduction study.

O. Oncogenicity

Due to the evidence for mutagenic and teratogenic potential of chlorfenvinphos, we require a repeat of the two-year feeding study with rats. The study should include 50 animals of each sex at each of 3 dosage levels in addition to the control group. All groups are to be from a viable colony of rats. The highest dose tested should be the MTD--the highest dose predicted not to significantly reduce longevity due to effects other than tumors. The MTD may or may not produce slight pharmacological effects or slight effects on weight gain. The chronic feeding study (rats; Access No. 090840, Vol 2, Part I, Sec 27) will not satisfy this requirement due to the unsatisfactory survival rate (control survival--18% female, 48% male). The dog study (Access No. 090840 Vol 2, Part I, Sec 30) will not satisfy this requirement since it is not a life-time study.

We also require an oncogenicity study in one mammalian species other than the rat.

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P. Antidotes

Adequate antidotal information is in the literature and conforms to typical suggestions for organophosphates. Atropine and oximes are antidotal. Some clinical diagnostic information also exists. PIMS file requested 28 Feb. 78.

HED:TS-769: D.G. Van Ormer D G Van Ormer

init: gjl: Reto Engler 7/20/78