UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorpyrifos Cholinesterase Studies in the Rat and in Man. Tox. Chem. No. 219AA

TO: Jay Ellenberger
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Review of Data:

1. Pilot Cholinesterase Study (Oral and Dermal), Rats. Conducted and submitted by Dow Chemical Co., Midland MI, Final Report dated December 4, 1981.

Animals used on test were 12 week old Fischer 344 rats supplied by Charles River Breeding Laboratories. Animals were acclimated for two weeks prior to test.

Test material used was analytical grade chlorpyrifos (Lot No. AGR 166043) with a purity of 99.8%. Stock solution was prepared by dissolving 1.00 g of test material in 5 ml of methylene chloride. A further dilution with corn oil was made by dissolving 100 microliters of stock solution in 10 ml of corn oil. For dermal exposure additional methylene chloride was added to dilute to 10, 40 and 160 mg/ml.

A total of 3 rats were dosed orally at the level of 5 mg/kg and two animals were orally dosed with an unspecified amount of corn oil. Three animals were also dermally dosed at levels of 5, 20 and 80 mg/kg and two control animals received dermal dosing to unspecified amounts of methylene chloride. Dermal exposure was to the clipped skin of the upper back. Animals were killed 24 hours after dosing.

Two additional animals were given a 5 mg/kg oral dose and two more animals were given a single 20 mg/kg dermal dose. These animals were killed 4 hours after treatment.

Prior to sacrifice, all animals had blood collected by cardiac puncture.

Chlorpyrifos and 3,5,6-trichloro-2-pyridinol levels and plasma and erythrocyte cholinesterase activities were determined in blood.

Results:

The following table is taken from p. 7 of the study report and summarizes all ChE findings of the study.

Table 1. Plasma and Erythrocyte Cholinesterase Levels in Male Fischer 344 Rats 24 Hours After A Single Oral Or Dermal Dose of Chlorpyrifos.

	Oral		Dermal			
Dose (mg/kg)	0	5	0	5	20	80
Plasma Cholinesterase	6.90 ±0.42	4.80 ±0.17	7.00 ±0.57	4.50 ±0.10	1.53 ±0.23	0.66 ±0.30
Erythrocyte Cholinesterase	8.45 ±2.19	4.57 ±1.30	11.10 ±0.57	10.57 ±2.83	1.87 ±1.25	3.93 ±1.37

Values represent one tenths of an international unit per ml, and are expressed as mean ± S.D. for 2 rats at the 0 dose level and 3 rats at the other dose levels.

These data indicate plasma cholinesterase inhibition at all oral and dermal dose levels. Erythrocyte cholinesterase inhibition was not observed at a dermal dose level of 5 mg/kg but was observed at 20 and 80 mg/kg. Clear inhibition of RBC ChE was observed at the oral dose level of 5 mg/kg.

Blood concentrations of Chlorpyrifos were 20 and 35 ng/ml after the oral dose of 5 mg/kg and the concentration of the TCP metabolite was 4210 ng/ml in the animal for which a measurement available. After the dermal exposure of 20 mg/kg, blood levels of Chlorpyrifos after 4 hours were 14 and 11 ng/ml and the TCP metabolite were 418 and 867 ng/ml.

Core Classification:

Supplementary Data. Too few animals were used for this study to be definitive either as a metabolism study or a study establishing a NOEL for ChE inhibition. It appears that plasma ChE inhibition is observed after an oral dose of 5 mg/kg and after dermal dosing at 5 mg/kg (LDT). RBC ChE inhibition was observed after an oral dose of 5 mg/kg and dermal doses of 20 mg/kg and greater.

2. Cholinesterase and Pharmacokinetics (Oral and Dermal), Humans. Conducted and submitted by the Dow Chemical Co., Final Report dated August 1982.

A total of 6 adult male Caucasians were selected for this study by public advertisement and screening by a physician for good general health status. The selected subjects were reported not to have had recent exposure to anticholinergic agents or chronic medication. Subjects were requested to refrain from aspirin, alcohol and all drugs for 24 hours before and after each dose.

The test material used was analytical grade Chlorpyrifos (Lot No. AGR 166043), purity of 99.8%. The oral doses were administered by dissolving the Chlorpyrifos in methylene chloride and transferring an appropriate amount of the mixture of a 0.5 gram lactose tablet. After evaporation of the methylene chloride, the tablet was taken with 100 ml of H_2O. Chemical analysis of duplicate tablets and weighing of tablets before and after addition of the test material was used to verify dose. Dermal doses were dissolved in either methylene chloride or DOWANOL DPM (dipropylene glycol methyl ether) and were placed on the volar surface of the forearm.

One subject served as a pilot in this study in the sense that he was administered dosages prior to the other 5 members of the group. The 0.5 mg/kg oral dose was administered one month prior to the other subjects. At the time that the other subjects were administered their oral doses, the pilot subject was administered a dermal dose of chlorpyrifos dissolved in methylene chloride; two weeks later this subject was given the same dermal dose dissolved in DOWANOL DPM. All other subjects were administered dermal doses of 5 mg/kg dissolved in DOWANOL DPM.

All dosing occurred between 8:30 and 9:30 A.M., about 30 minutes after the consumption of "a standard breakfast". Dermal doses were allowed to freely evaporate and subjects followed normal bathing practices.

Urine samples were collected, when available, both 2 days prior to and 4-5 after dosing. In addition, separate collections of urine were made at 0, 6, 12, 24, 36, 48, 60, 72, 96, 156 and 180 hours after dosing (the later intervals were only for the 5 mg/kg dermal dose). Urine volume and creatinine concentrations were measured at each interval and urine chlorpyrifos and 3,5,6-trichloro-2pyridinol using the modified method of McKellar.

Blood samples were collected at 35 intervals ranging one hour to 60 days after the initial oral dose. Samples were analyzed for plasma and erythrocyte cholinesterase, chlorpyrifos and 3,5,6-trichloro-20pyridinol concentrations.

Results:

No signs or symptoms of toxicity were observed at any time.

Mean plasma cholinesterase determinations are shown in the following table (taken from p. 18 of the registrants submission):

Post Oral Dose (Days)	Mean Plasma ChE Activity (pH units/hr)
Predosing	1.16
.08	.84
.25	.79
.5	.21
1	.17
2	.26
3	.31
4	.39
8	.54
14	.84
22	.86
27	1.03
30	.98

Thus a clear depression of plasma ChE was observed with levels reduced to their maximum extent after one day (15% of predosing activity). Although erythrocyte cholinesterase levels were not clearly depressed after administration of 0.5 mg/kg oral dose, the small number of subjects and the wide fluctuations in individual activity levels prevented the determination of whether this was the NOEL for erythrocyte ChE inhibition.

Dermal doses of 5 mg/kg elicited no obvious effect on either plasma or erythrocyte ChE, but again the small number of subjects and the variability of the data prevented the determination of a NOEL.

The data gathered was used to solve the following equation:

$$C_b = \frac{K_a \times \text{Dose} \times F}{V_d \times (K_a - K_e)} \times e^{-(K_e \times t - K_a \times t)}$$

Where C_b = blood [TCP]
F = Fraction of dose absorbed
V_d = Volume of Distribution
K_a = 1st order rate constant for absorption
K_e = 1st order rate constant for elimination
T = Time

The volume of distribution was calculated to be 15.1 liters, the elimination rate constant was calculated to be .0258 hr and the elimination half-life to be 26.9 hrs. Because blood chlorpyrifos levels were much lower than TCP levels (mean levels of chlorpyrifos were at least 90 fold less than mean TCP levels throughout the first 12 hours after dosing) and only TCP was found in urine, it appeared that chlorpyrifos is rapidly metabolized to TCP. The model indicates 72 ± 11% of the oral dose was absorbed and excreted as TCP and this compares well with the 70 ± 11% of the oral dose recovered in the urine as TCP over the 8 days of the metabolism study. When the data for the oral phase of the study are considered in toto, they indicate that chlorpyrifos is well absorbed via the oral route of administration, that the parent compound is rapidly metabolized to TCP, and that the majority of the TCP metabolite is rapidly (within 8 days) excreted in the urine. The excretion appears to follow first order kinetics at the dose levels administered in this study (.5 mg/kg oral, 5 mg/kg dermal).

The dermal exposure indicates a slower rate of absorption than the oral route (blood levels peak at 24 hours after dermal exposure vs. 6 hours after oral exposure). Although only about 3% of the dermal dose appears to have been absorbed, the effect of the vehicle and other experimental conditions can not be determined. The rates of metabolism and excretion after dermal exposure are similar to those observed after oral exposure with only a slightly longer excretion half-life.

Core Classification:

Supplementary Data. The small number of subjects and the variability of the data prevent the determination of a definitive NOEL for ChE inhibition based on this study. A clear effect on plasma ChE is observed after an oral dose of 0.5 mg/kg/day.