5-23-84

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003822

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Jay Ellenberger, PM #12
Insecticide-Rodenticide Branch
Registration Division (TS-769)

THRU: William L. Burnam, Chief
Toxicology Branch/HED (TS-769)

W.L.Burnam 5-23-84

SUBJECT: Chlorpyrifos Registration Standard

Submission of the Toxicology Branch evaluation of Chlorpyrifos toxicity data for registration standard purposes consists of the following:

1. Review of toxicity data for chlorpyrifos including bibliography

2. Updated TOX "One-liners"

3. Data Summary Table A which indicates TOX data gaps

4. Policy discussion, tolerance assessment and ADI re-evaluation

Gary J. Burin

Gary J. Burin, Toxicologist
Review Section V
Toxicology Branch/HED (TS-769)

1891

Chlorpyrifos

Acute

Acute oral, dermal, eye irritation and dermal irritation studies are available (see attached "One-Liners"). These studies indicate that chlorpyrifos labeling should be either Tox. Cat. II (dermal and oral LD_{50}) or Tox. Cat. III (eye and dermal irritation). An inhalation acute LC_{50} study is not available and is required.

Neurotoxicity

The potential of chlorpyrifos to induce acute delayed neurotoxicity was first investigated in 1966 (1). That study was classified as Invalid and an additional neurotoxicity study was submitted in 1978 (2). Hens were administered atropine and either tri-o-tolyl phosphate (positive control), chlorpyrifos at 50 mg/kg or 100 mg/kg or atropine alone. Neither clinical nor histological signs of acute delayed neurotoxicity were observed in chlorpyrifos treated birds whereas positive control animals exhibited the expected response. The study demonstrated that chlorpyrifos is not an acute delayed neurotoxic agent at dosages up to 100 mg/kg.

Subchronic, Chronic and Oncogenicity

The most sensitive endpoint for assessment of subchronic or chronic toxicity after exposure to chlorpyrifos is clearly AChE inhibition and its sequellae. Other non-specific indications of toxicity occur only at dose levels several orders of magnitude greater than signs of AChE inhibition. For example, the chronic dog study found significant RBC and plasma ChE inhibition at dosages as low as 0.03 mg/kg/day but an increase liver weight and liver/body weight ratio only at a dosage of 3 mg/kg/day (3). No other toxic effects were observed at levels up to and including 3 mg/kg/day. However due to study deficiencies such as inadequate reporting of histology data, and lack of supporting data for some intervals for parameters such as body weight and ophthalmology, this study is not adequate for the evaluation of the toxicity of chlorpyrifos.

The chronic rat study of chlorpyrifos was similarly limited by inadequate reporting of histology, clinical observations and body weights (4). This study was also classified as Supplementary Data. However, compound-related effects other than ChE inhibition were not observed in this study at dose levels up to and including 3.0 mg/kg/day.

Although neither the chronic rat study nor the mouse oncogenicity studies indicate an oncogenic potential for chlorpyrifos, the rat study is of limited value (as noted above) and the mouse oncogenicity study may not have been conducted using a maximum tolerated dose (20). Additional information is being requested to determine whether the appropriate doses were tested in the mouse oncogenicity study.

A 180 day study of chlorpyrifos in rhesus monkeys found no compound related effects, other than ChE inhibition, at dose levels up to and including 2 mg/kg/day (5). This study was limited by the small number of animals used at each dose level (2 males and 2 females for the control and high dose groups, 2 males and 1 female for the low and mid dose groups.) It has been classified as Supplementary Data.

Human Toxicity

Two human studies are useful in assessing the toxicity of Chlorpyrifos. The first study used a single oral dose of 0.5mg/kg and a dermal dose of 5 mg/kg (6). Plasma but not RBC cholinesterase was depressed after oral dosing and neither plasma nor RBC ChE was depressed after dermal dosing. The second study exposed human volunteers to levels of either 0.10, 0.030, or 0.014 mg/kg for periods ranging from 9-27 days (7). The Lowest Observed Effect Level (for plasma ChE depression and clinical toxicity) was 0.10 mg/kg and the NOEL was 0.03 mg/kg.

Mutagenicity

Acceptable mutagenicity studies of chlorpyrifos are not available. The mutagenicity studies that have been submitted have been classified as Unacceptable (8,9,10,11).

Teratology and Reproduction

Teratogenicity of chlorpyrifos was initially investigated as part of a rat reproduction study (12). The F_2 generation parents had been fed dietary dosage levels of 0, 0.1, 0.3 and 1.0 mg/kg (20 females per dose level) and on days 6-15 of gestation were administered these dose levels by gavage. Dietary administration was resumed after day 15. On day 20 females were sacrificed and fetuses were examined. Pup mortality was slightly increased at the high dose level but no other aspects of fetotoxicity were observed. Aside from ChE inhibition in the dams at all but the low dose level, maternal toxicity was not observed in this study.

The reproduction study itself was not conclusive with respect to a NOEL for reproductive effects due a high background rate of pup mortality. No teratogenic effects were observed at any dose level. The study (excluding the teratology phase) was recently repeated and no reproductive effects were observed at dose levels up to 1.2 mg/kg/day, the highest dose tested (13).

A teratology study in mice (Core Minimum data) showed no teratogenic effects at dose levels up to and including 25 mg/kg/day (highest dose tested) (14). A recently submitted teratology study conducted in the rat was classified as Core Minimum data with no chlorpyrifos related effect on either soft tissue or skeletal development (15) . Maternal toxicity was demonstrated only at the high dose level (15 mg/kg). In addition to the above studies, embryonal toxicity studies in cows and chickens have been conducted. These studies,however, are considered unacceptable by the Agency.

Data Gaps

As noted above, the chronic dog and rat studies, the mouse oncogenicity study and the available mutagenicity studies are not adequate for regulatory purposes and must either be upgraded or, if this is not possible, repeated. In the case of the mutagenicity studies, additional studies should be conducted to satisfy the current Agency requirements. In addition, a metabolism study that satisfies the requirement in this area should be submitted.

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Requirement	Composition	Use Patterns[1][2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3]
3.135 Toxicology					
ACUTE TESTING:					
-1 - Oral LD_{50} - Rat	TGAI	A,B,D,F,H,I	Yes	Acc.No 112115	No
-2 - Dermal LD_{50}	TGAI	A,B,D,F,H,I	Yes	Acc.No 112115	No
- Inhalation LC_{50} - Rat	TGAI	A,B,D,F,H,I	No		Yes
-7 - Acute Delayed Neurotoxicity - Hen	TGAI	A,B,D,F,H,I	Yes	2	No
SUBCHRONIC TESTING:					
-1 - 90-Day Feeding - Rodent, Non-rodent	TGAI	na	na		No
-2 - 21-Day Dermal	TGAI	na	na		No
-3 - 90-Day Dermal	TGAI	na	na		No
-4 - 90-Day Inhalation - Rat	TGAI	H,I	No		Yes
-5 - 90-Day Neurotoxicity-Hen/Mammal	TGAI	na	na		No

Composition: TGAI = Technical grade of the active ingredient.
The use patterns are coded as follows: A=Terrestrial, Food Crop; B=Terrestrial, Non-Food; C=Aquatic, Food Crop; D=Aquatic, Non-Food; E=Greenhouse, Food Crop; F=Greenhouse, Non-Food; G=Forestry; H=Domestic Outdoor; I=Indoor.
Data must be submitted no later than _____.

:a Requirement	Composition [1]	Use Pattern [2]	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?
58.135 Toxicology (continued)					
CHRONIC TESTING:					
83-1 - Chronic Toxicity - 2 species: Rodent and Non-rodent	TGAI	A	Partially	3,4	Yes
83-2 - Oncogenicity Study - 2 species: Rat and Mouse preferred	TGAI	A	Partially	4,20	Yes
83-3 - Teratogenicity - 2 species	TGAI	A,B,D,F,H,I	Yes	12,14,15	No
83-4 - Reproduction, 2-generation	TGAI	A	Yes	12, 13	No
MUTAGENICITY TESTING					
84-2 - Gene Mutation	TGAI	A	No		Yes
84-2 - Chromosomal Aberration	TGAI	A	No		Yes
84-2 - Other Mechanisms of Mutagenicity	TGAI	A	No		Yes

a Requirement	1 Composition	2 Use Pattern	To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Data Be Submitted Under FIFRA Section 3(c)(2)(B)?[3]
8.135 Toxicology (continued)					
SPECIAL TESTING					
5-1 - General Metabolism	PAI or PAIRA	A	Partially		Yes
5-2 - Domestic Animal Safety	Choice	H,T	Yes		No

§158.155 TOXICOLOGY
(continued)

1. Composition: PAI = Pure active ingredient; PAIRA = Pure active ingredient, radiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.
2. The use pattern(s) are coded as follows: A=Terrestrial, Food Crop; B=Torrestrial, Non-Food; C=Aquatic, Food Crop; D=Aquatic, Non-Food; E=Greenhouse, Food Crop; F=Greenhouse, Non-Food; G=Forestry; H=Domestic Outdoor; I=Indoor.
3. Data must be submitted no later than _____.

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Chlorpyrifos Tolerance Reassessment

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Chlorpyrifos [0,0-Diethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is an organophosphate pesticide that is rapidly absorbed following oral administration and is rapidly metabolized to 3,5,6-trichloro-2-pyridinol and 3,5,6-trichloro-2-pyridyl phosphate. The parent compound is excreted primarily in the urine with an elimination half-life of 26.9 hrs (16). Chlorpyrifos has not been shown to be neurotoxic, oncogenic or teratogenic in the available tests.

The primary toxicological concern associated with chlorpyrifos is the inhibition of acetylcholinesterase. Signs of acetylcholinesterase inhibition are manifested by muscarinic and nicotinic effects and include constricted pupils, watery eyes, anorexia, nausea, vomiting, diarrhea and salivation (17). Poisoning may progress to respiratory depression, convulsions and deaths.

The Allowable Daily Intake for chlorpyrifos is based on the AChE inhibiting property of chlorpyrifos. Species differences exist with respect to sensitivity to this effect. The inhibition of plasma AChE sometimes also appears to be more sensitive than the inhibition of blood or brain AChE. However, in the two year chronic rat study, the rat teratology study, the two year dog study and the 180 day monkey study, the same NOELs are observed for both RBC and plasma ChE inhibition. The lowest No Observed Effect Levels and Lowest Observed Effect Levels for cholinesterase inhibition are as follows for the rat, dog, monkey and man:

Study	Results		Core Classification	Reference
	NOELs	LELs		
Rat				
180 Days	0.15 mg/kg	0.75 mg/kg	Supplementary	5
2 Years	0.1 mg/kg	0.3 mg/kg	Supplementary	4
3 Generation	Plasma			
	0.1 mg/kg	0.3 mg/kg	Minimum	12
	RBC			
	1.0 mg/kg	1.0 mg/kg		
Rat Teratology	0.1 mg/kg	0.3 mg/kg	Minimum	15
Dog				
2 Years	0.01 mg/kg	0.03 mg/kg	Supplementary	3
Monkey				
180 Days	.08 mg/kg	0.4 mg/kg	Supplementary	5
Man				
	Plasma			
9-20 Days	0.03 mg/kg	0.1 mg/kg	Supplementary	7
	RBC			
	0.1 mg/kg	0.1 mg/kg		

The relevance of each of the ChE determinations (plasma, RBC and brain) to human health has been the subject of a great deal of scientific discussion. Only ChE inhibition at the synapse is directly relevant to the human health; however measurement at the synapse is only measured in the assay of brain ChE in routine toxicological studies. Because cholinergic synapses are distributed throughout the peripheral nervous system as well as in the brain, and because the distribution of a chemical to the brain is often quantitatively different than that to other tissues due to differences in capillary permeability (often referred to as the "blood-brain barrier" although there is in fact no real evidence for an absolute barrier (18)), the inhibition of brain ChE may not be the most sensitive indicator of potential anti-cholinergic effects resulting from exposure to AChE inhibitors.

A recent WHO monograph (1982) stated erythrocyte (RBC) cholinesterase inhibition may be a better indicator of the biochemical effect of anti-ChE pesticides than plasma ChE because RBC ChE is thought to be biochemically identical to ChE at the synapse whereas plasma ChE is considered to be quite different (19). However, it is known that brain ChE may be inhibited in the absence of either RBC or plasma ChE inhibition and the pattern of inhibition thus appears to vary from one anti-ChE agent to another.

Measurement of chlorpyrifos-induced plasma and RBC cholinesterase inhibition often finds that plasma cholinesterase inhibition is a more sensitive indicator of anti-ChE activity than that of RBC or brain cholinesterase inhibition although the relevance of plasma ChE inhibition to human health has not been clearly established. However, as previously noted, the NOELs for plasma and RBC inhibition are the same in several of the chlorpyrifos studies and in the chronic rat study the percent inhibition of ChE at the LOEL is clearly greater for RBC than for plasma.

For chlorpyrifos, marked inhibition of plasma ChE (in the absence of RBC ChE inhibition) coincides with the level at which the classic clinical signs of ChE inhibition are noted <u>in a study in humans</u>. The recently received human cholinesterase study demonstrates a Lowest Observed Effect Level of 0.1 mg kg/day based on 9 days of exposure. The NOEL in this study is 0.03 mg/kg/day (based on 20 days of exposure at this level .

The ADI for chlorpyrifos had previously been based on plasma and RBC ChE inhibition in a 2-year rat feeding study (NOEL = 0.1 mg/kg/day). It is now recommended that the ADI for chlorpyrifos be set at 0.003 mg/kg/day based on a study in humans with a NOEL of 0.03 mg/kg/day for ChE inhibition and a 10-fold safety factor. It should be noted that the duration of exposure was quite short in the human study and a progression of the effect through time was observed. It should also be noted that the number of subjects

12

was small (4 per dose level) and the sensitivity of the test is therefore limited. Furthermore, only males were tested. However, it has been the policy of the Agency to use data from human studies in those rare cases where they are appropriate and available and, in this case, the human data is similar to the animal data (yielding a NOEL that is one-third of the NOEL in rats and three times that of the NOEL in dogs for ChE inhibition).

The Theoretical Maximum Residue Level (TMRC) utilized 105% of the previous ADI. The TMRC (including recently published tolerances) will utilize approximately 350% of the ADI. Dietary exposure to chlorpyrifos may be associated with the chlorpyrifos metabolite (3,5,6-trichloropyridinol) recently found in the urine of 5.8% of individuals sampled in the Health and Nutrition Examination Survey (HANES II) conducted by the National Center for Health Statistics. The actual source of the metabolite found in this survey is presently unknown.

The tolerance expression currently includes the 3,5,6-trichloropyridinol (TCP) metabolite which does not inhibit cholinesterase. Comparison of residues of TCP (reflected in the TMRC) with an ADI that is established based on ChE inhibition may overestimate the hazard associated with the use of the parent compound (chlorpyrifos). For example, if the dietary residues resulting from the use of chlorpyrifos were mainly the TCP metabolite and this metabolite was much less toxic than the parent compound, the comparison of a Theoretical Maximum Residue Concentration which included TCP to the ADI would have little relevance. On the other hand, if exposure is primarily to the parent compound a separate tolerance for TCP would make little sense. Although at this time there is insufficient residue and toxicity information to exclude TCP from the tolerance expression, it is recommended that an attempt be made to better elucidate both toxicity and exposure to the TCP metabolite with the intention of either reconsidering the inclusion of the TCP metabolite in the tolerance expression or expressing the TCP tolerance separately from that of the parent compound.

CFR 180.342 ● Chlorpyrifos ● 5/13/84

File last updated 5/14/84

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NOEL change not recorded!

ACCEPTABLE DAILY INTAKE DATA

human mg/kg	NOEL ppm	s.f.	ADI mg/kg/day	MPI mg/day(60kg)
0.030	3.00	10	0.0030	0.1800

Published Tolerances

Crop	Tolerance	Food Factor	mg/day(1.5kg)
Bananas(7)	0.050	1.42	0.00107
Corn,all types(36)	0.100	2.51	0.00377
Cottonseed (Oil)(42)	0.500	0.15	0.00112
Eggs(54)	0.100	2.77	0.00416
Hogs(65)	0.300	3.43	0. 2575
Milk&Dairy products(98)	0.025	23.52	0.00558
Peaches(114)	0.050	0.90	0.00067
Pears(122)	0.050	0.10	0.00015
Plums,not prunes(144)	0.050	0. 9	0. 0007
Sweet potatoes(167)	0.050	0.40	0.00060
Sorghum(174)	0.150	0. 3	0.00034
Broccoli(19)	0.050	0.10	0.00007
Cabbage,Sauerkraut(22)	0.050	0.74	0. 2207
Cauliflower(27)	0.050	0.07	0.00215
Rutabagas(180)	0.050	0.15	0.00135
Tomatoes(163)	0.500	4.87	0.02156
Walnuts(200)	0. 05	0.15	0.00135
Nectarines 000	0.050	0. 3	0.00002
Brussel sprouts(00)	0.050	0. 3	0.00093
Peanuts(118)	0.050	0.30	0. 0269
Cucumbers,Inc pickl(46)	0.200	0.73	0.00109
Pumpkin,Inc squash(000)	0.050	0.11	0.00017
Seedcrop veg(140)	0.050	0.60	0. 0849
Mint(132)	0.050	0.03	0.00045
Turnips(183)	0. 05	0.05	0. 0230
Turnip greens(180)	0.050	0.03	0.00045
Chinese Cabbage(177)	0.050	0.03	0. 0090
Peppers(000)	0.050	0.22	0.00164
Prunes(138)	0.050	0.14	0.00003
Goats(62)	0.050	0.03	0.00045
Sheep(140)	0.050	0.15	0.00091
Poultry(000)	0.500	0. 9	0. 2207
Horses(000)	0. 05	0.03	0.00045
Strawberries(152)	0.050	0.09	0.00135
Soybeans Oil,(148)	0.050	0.03	0.00039
Cattle, 00	0.050	0.15	0.00553
Onion(Gr, Bulb,(000	0.050	0. 2	0.00037
Sunflower(150)	0.050	0.10	0.00011
Citrus fruits(30	0.050	0.21	0.00714
Grapes, not raisins(87)	0.050	0.05	0. 0337
Cherries(30)	0.050	0.10	0.00007
Figs(87)	0.050	0.03	0.00005
Cranberries(44)	0. 05	0. 3	0.00045
Apples(00	0. 05	0.03	0. 5603
Sugar,Sugarbeet,000	0. 05	0. 3	0. 5457

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Almonds([redacted]	0.200	0.03	[redacted].00009
Walnuts(167)	0.200	0.03	0.00009
Asparagus(5)	5.000	0.14	0.01073
Kiwi Fruit(204)	2.000	0.03	0.00090

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ADI	MRC	% ADI
0.1800 mg/day(60kg)	0.5637 mg/day(1.5kg)	313.15

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Unpublished, Pox Approved 152436,352819,352886

Crop	Tolerance	Food Factor	mg/day(1.5kg)
All Foods(197)	0.025	100.00	0.03750
Wheat(170)	0.155	10.36	0.02332
Collards(57)	2.000	0.03	0.00245
Kale(75)	2.000	0.03	0.00090
Kohlrabi(76)	2.000	0.03	0.00090
Mustard Greens(99)	2.000	0.06	0.00134
4 Brassica leafy vegs (214)	2.000	0.03	0.00090
Mushrooms(97)	0.100	0.03	0.00002

ADI	MRC	% ADI
0.1800 mg/day(60kg)	0.6315 mg/day(1.5kg)	350.83

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☆ see 3-29-83 printout

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: The File

FROM: William Burnam, Chief
Toxicology Branch

WJB 5-18-84

SUBJECT: Chlorpyrifos Mouse Oncogenicity Study
TOX Chem #219AA

There is very limited evidence to indicate that the highest dose tested (15 ppm) was high enough to cause any toxicological effect or be considered the maximum tolerated dose. Data on body weight, food consumption, mortality and clinical observations showed little or no dose related toxic effects of chlorpyrifos. Data on ACh values were not taken during the study. There apparently was no pilot study or subchronic oral study to determine the MTD of chlorpyrifos in the mouse.

At the present time we should change our classification of this study to Supplementary but the registrant should be informed that ancillary data should be forth coming indicating the justification and suitability of the doses used.

CAS 219AA
3F2947
3H5411
Accession 071866

Chlorpyrifos: Oral Teratology Study on Fischer 344 Rats

by Oielette, J. H., Dettenber, D.A., Kloes, P.M., and John, J. A.

Tox. Research Lab, Dow Chemical, Midland, Mich. July 5, 1983.

Materials and Method:

Test material: Dow Chemical Chlorpyrifos 96.6% pure

Compound Prep & Dosing: Chlorpyrifos was dissolved in corn oil and given by gavage at a level of 4 ml/kg.

Animals: Male and female Fischer 344 rats were acclimated for 2 weeks then bred (one to one). Day zero was the day sperm was found in a vaginal smear. Females were randomized into dose groups.

Design:

Dose mg/kg/day	0	0.1	3.0	15.0
Number females	31	31	32	33

Dosing was carried out daily on days 6-15 of gestation.

Observations

The following observations were carried out on the dams and fetuses according to pp 5 and 6 of the report of the authors.

> "Maternal Observations: Animals were observed daily throughout the experimental period for indications of toxicity from the test material. Body weights for rats were recorded on gestation days 6 through 16 and on day 21 of gestation; food and water consumption were recorded for each rat at 3-day intervals starting on day 6 of gestation. Statistical analysis of body weight and body weight gain was performed using data recorded on days 6, 9, 12, 16, and 21 of gestation. In addition, the maternal liver weight was recorded at the time of Cesarean section on day 21 of gestation. Separate groups of 10 bred rats/dose level were dosed on days 6 through 15 of gestation and sacrificed following methoxyfluorane anesthesia on day 15 of gestation (approximately 4 hours after dosing). Maternal blood was obtained by cardiac puncture for determination of plasma and erythrocyte cholinesterase levels.[3]

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Fetal Observations. Test animals were sacrificed by carbon dioxide inhalation on day 21 of gestation. The uterine horns were exteriorized through a mid-line abdominal incision. The following data were recorded: 1) number and position of fetuses in utero, 2) number of live and dead fetuses, 3) number and position of resorption sites, 4) the number of corpora lutea, 5) the sex, body weight and crown-rump length of each fetus, and 6) any gross external alteration. The uteri of apparently non-pregnant animals were stained with a 10% solution of sodium sulfide (Kopf, et al., 1964) and examined for evidence of implantation sites. This procedure was done solely to determine the incidence of pregnancy and resorptions observed by staining were not used in resorption rate calculations. One-half of each litter, selected using a table of random numbers, was examined immediately by dissection under a low power microscope for evidence of soft tissue alterations (Staples, 1974). The heads of rat fetuses examined by dissection were removed, placed in Bouin's fixative and examined by the serial sectioning technique of Wilson (1965). All fetuses were then preserved in alcohol, eviscerated and subsequently cleared and stained with alizarin red-S (Dawson, 1926) and examined for skeletal alterations."

Statistics

The authors report on pp 6 and 7 of their report that the following statistical analyses were performed.

"Body weight, food and water consumption, hematological parameters, and absolute and relative organ weights were evaluated by Bartlett's test for equality of variances. Based upon the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was performed followed by Dunnett's test or followed by the Wilcoxon Rank-Sum test with Bonferroni's correction (Steel and Torrie, 1960) if the ANOVA was significant. Statistical evaluation of the frequency of pre-implantation loss and of resorptions among litters and the fetal population was made by a censored Wilcoxon test (Haseman and Hoel, 1974) with Bonferroni's correction. Other incidence data were analyzed by the Fisher exact probability test (Siegal, 1956).

3 Plasma and erythrocyte cholinesterase determined by a modification of the Boehringer Mannheim/Corporation colorimetric technique, (Shaumber, Illionis)

The nominal alpha levels used are as follow:

Bartlett's Test for Variances	α =0.01
Analysis of Variance	α =0.10
Dunnett's Test	α =0.05 two-sided
Wilcoxon Rank-Sum Test	α =0.05 two-sided, with Bonferroni correction (Miller, 1966)
Fisher's Test	α =0.05 one-sided
Censored Wilcoxon Test	α =0.05 one-sided
Grubbs Outlier	α =0.02

Because numerous measurements are statistically compared in the same group of animals, the overall false positive rate (Type of I errors) is much greater than the cited alpha levels would suggest. Thus, the final interpretation of numerical data considers statistical analyses along with other factors such as dose-response relationships and whether the results are significant in the light of other biologic and pathologic findings."

Results

Maternal

Although there are no summary sheets or individual animal data sheets to indicate this the authors state that the highest dose (15.0 mg/kg) caused excessive salivation; tremors and other effects typical were noted in the other groups. On certain days (12 and 16), there was less weight gain by the only high dose females.

There were no dose related effects on the liver wt or food consumption and minor effects at the high dose on water consumption.

Clear evidence of cholinesterase inhibition in the plasma and RBC were seen at the 2 highest doses but not in the low dose. Therefore the NOEL for both plasma and RBC cholenesterase inhibition in the study was 0.1 mg/kg/day.

Fetal Toxicity

There were no significant effects on litter size, resorption rate, fetal body wt or length except that in the 2 high doses the fetal wts were increased above control. This increase was not dose related since the effect was more evident at 3.0 than at 15.0 mg/kg/day.

Fetal Anomalies

Various malformations or anomalies, were seen in all groups without any apparent dose-response relationship. There

was no chlorpyrifos related effect on either soft tissue or skeletal development.

Conclusion: Chlorpyrifos was not teratogenic or fetotoxic up to 15.0 mg/kg/day which was a dose which caused cholinergic signs of toxicity in the dams. These high dose dams also gained a little less wt and consumed less water. In a separate satellite study, AChE depression (RBC and plasma) was seen at 3.0 and 15.0 mg/kg/day but not at 0.1 mg/kg.

NOEL (maternal toxicity)	=	3.0 mg/kg (systemic)
NOEL (maternal toxicity)	=	0.1 mg/kg (AChE, plasma and RBC)
NOEL (teratogenic and fetotoxic)	=	15.0 mg/kg HDT

Classification: Core Minimum. The appendix should contain information on the time of onset of signs of toxicity and their degree of severity and duration, however other individual data on dams and pups are presented in sufficient detail.

W A Burnam
5-18-84

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003822

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Use of Mouse Teratology Study in Chlorpyrifos Registration Standard CASWELL#219AA

TO: FILE

FROM: Gary J. Burin, Toxicologist
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769)

I have examined the November 13, 1979 review of this study by William Dykstra of Toxicology Branch and compared it to the final report. I agree with the original conclusions of the prior Toxicology Branch review that chlorpyrifos is not teratogenic at doses up to 25 mg/kg/day and that the NOEL for ChE inhibition is 0.10 mg/kg/day in this study. I also agree with the study classification (Core-Minimum).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003822

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Use of Acute Delayed Neurotoxicity Study (Accession No. 097144) for the Chlorpyrifos Registration Standard. CASWELL#219AA

TO: THE FILE

FROM: Gary J. Burin, Toxicologist
Toxicology Branch

I have compared the final report for this study (dated May 22, 1978) with the Toxicology Branch review of 9/3/81 by William Dykstra. I agree with conclusion that chlorpyrifos is not a delayed neurotoxic agent at doses of up to 100 mg/kg (HDT) and that study was adequate for the purpose of detecting acute delayed neurotoxicity (Core-Minimum Data).

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003822

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Human Cholinesterase Study
Tox. Chem. 3194A
TO: Jay Ellenberger, PM#12
Registration Division (TS-769)

FROM: Gary J. Burin, Toxicologist GB 5/18/84
Section 7, Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Recommendation: It is recommended that this study be classified as Supplementy Data. Although the number of subjects in this study is small (4) and only males were tested, a LEL for plasma ChE of 0.10 mg/kg/day is indicated which is accompanied by clinical signs of ChE inhibition. The apparent NOEL is 0.03 mg/kg/day. RBC ChE inhibition is not observed at any dose level. It is also recommended that this study, despite its limitations, play important role in the establishment of the ADI for chlorpyrifos.

Review of Data:

Three-Week Toxicity Study with Cholinesterase Determinations, Humans. Conducted by Albany Medical College, Albany, New York, March 1972 and submitted by Dow Chemical, April 6, 1984.

Sixteen human volunteers were selected from the Clinton Correctional Facility in Dannemora, N.Y. and were subjected to a thorough physical examination which included ECG, urinalysis, chest x-ray, urinalysis, hematology and clinical chemistry.

The volunteers were (apparently randomly) divided into 4 experimental groups which received daily doses of either 0, 0.10, 0.030, or 0.014 mg/kg of body weight of Dowco 179 (purity not specified) via oral ingestion of the test material "in tablet form". Four individuals were assigned to each group. The tablets were administered once each day at the time of breakfast. Blood samples were taken twice each day from each volunteer for cholinesterase measurements and weekly for hematology and clinical chemistry measurements. Urinalysis was also conducted on a weekly basis. Treatment continued daily for a 7 week period for the control group, 3 days for the 0.10 mg/kg/day group, , 20 days for the 0.03 mg/kg day and 27 days for the 0.014 mg/kg/day dose group.

Results:

Clinical signs of cholinesterase inhibition were observed in one volunteer at the high dose (0.10 mg/kg). This individual reported having a "runny nose, blurred vision, and a feeling of faintness" after 9 days of treatment. Dosing of all individuals at the high dose was terminated after 9 days on test, apparently due to these clinical signs of toxicity and marked cholinesterase inhibition (see discussion of cholinesterase inhibition below).

Clinical chemistry and hematology were unremarkable. Although a slight elevation in serum glucose was observed in all groups (including the controls) compared to baseline values this is probably due to blood samples being taken soon after the consumption of a meal.

A clear depression of plasma cholinesterase was observed at the high dose level (0.10 mg/kg) with the group mean value for the group decreasing to 1.7 umoles acetate/min/ml from a mean baseline value of 4.75 (a decrease of 64%) on the nineth day of treatment. After day 9, treatment ceased and a gradual recovery was observed with ChE values equaling baseline values by day 25 of the recovery period.

At the next lowest dose level, 0.03 mg/kg, an equivocal depression of plasma ChE was observed with the mean value decreasing to 3.4 umoles acetate/min/ml from a mean baseline value of 4.7. Although this represents a decrease of 28%, the small number subjects (4) and the great amount of variation from one measurement to another precludes the determinations that 0.03 mg/kg is an effect level. Treatment at this level continued for 20 days compared to 9 days at the 0.10 mg/kg dose level.

No compound related effect on RBC cholinesterase could be associated with chlorpyrifos administration at any dose level although it is noted that the sensitivity of the study to detect an effect on RBC ChE is limited by the small numbers of subjects and the variability in the assay.

No compound related effect of any form was observed at 0.014 mg/kg/day and no effect was observed on RBC cholinesterase at any dose level.

Discussion:

The clinical signs reported at the high dose level of this study conform to expected signs of generalized toxicity resulting from an anti-AChE agent (Koelle, 1975). The runny nose and blurred vision may be considered muscarinic effects and the "faintless" is considered to be a nicotinic effect. Plasma cholinesterase at the high dose was inhibited 64% compared to baseline values and although an equivocal depression of plasma ChE was observed at the mid dose level (0.03 mg/kg), this could not be clearly classified as a treatment-related effect. The LEL for plasma ChE inhibition and clinical signs of toxicity is 0.10 mg/kg and the NOEL appears to be 0.03 mg/kg in this study.

Core-Classification: Supplementary Data. Only males were tested and the number of subjects (4 per dose level) limits the sensitivity of the test.

Koelle, G.B. Anticholinesterase Agents, in "The Pharmacological Basis of Therapeutics", L.S. Goodman and A. Gilman, eds. Macmillan Publishing Co., Inc., New York, 1975.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003822

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Jay Ellenberger, PM#12
Registration Division (TS-767)

FROM: Gary J. Burin, Toxicologist GB 5/18/84
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: PP#3F2947/3H5411/464-448.
Review of Chlorpyrifos Rat Reproduction Study
Tox. Chem. 213AA Acc. No.: 071367

Background Information:

This study was requested to help clarify the NOEL for reproductive effects in the rat. A previous study suggested an effect on neonatal survival at 1.0 mg/kg/day but was inconclusive due to a high background of neonatal mortality and great variability in the data. In a meeting held on 12/6/81 with representatives of the registrant, it was agreed that a new study would be undertaken. The study has been recently submitted and a review of that study follows:

Recommendation:

This study, standing alone, would be classified as Supplementary data. However, its intent was to establish a NOEL for neonatal survival and it is clearly acceptable for that purpose (NOEL = 1.2 mg/kg (HDT)). In combination with the previous reproduction study, the requirement for a Core Minimum Reproduction study is satisfied.

Review of Data:

Reproduction Study, Rats. Conducted and submitted by Dow Chemical Co., Freeport, Texas. Report dated July, 1983, report number not stated.

Sprague-Dawley rats of approximately 4 weeks of age were acclimated for 14 days prior to the initiation of the study. Animals were screened with regard to health status and animals at either

population of animals to be tested. Thirty male and thirty female animals were randomly assigned to groups which would eventually receive 0, 0.5, 0.8 or 1.2 mg/kg/day of test material. Animals were housed individually in stainless steel cages during all but the cohabitation, gestation and lactation phases, during which females were relocated to solid bottomed plastic reproduction cages with wood shavings for use as nesting material. Food and water were available ad libitum during the entire study.

Premixes were prepared every 1-3 weeks and test diets were prepared weekly. Premixes and test diet were analyzed 12 times during the course of the study. Test material was technical Dursban F (96.6% chlorpyrfios), Lot #811012-616. Reanalysis at the end of the test period indicated 98.6-99% chlorpyrifos.

The preparation of premix occurred in two stages. The first stage was originially with silica gel to a level of 1% a.i. and the second stage involved mixing this with test diet for a "working premix". Approximately two months into the F_0 generation the method of preparation of premix was changed to a direct mixing of test material with diet due to "a lack of analytically confirmable test material concentration in the test diets during the two weeks prior to 3/24/82". The study report further noted that "... a review of the available analytical data suggested that the extraction efficiency of the analytical method dropped considerably as the silica gel premix aged beyond 14 days." Although this reviewer could not verify whether the discrepancy between targeted and actual dietary levels was due to a difficulty with analysis or some other problem, it is noted that, aside from the analysis conducted on 3/17/82, actual and targeted concentrations in the diet were within 10% of each throughout the study. Therefore dietary levels of test could be confirmed for all but the two weeks preceding 3/24/82.

The concentration of test material in the diet was adjusted weekly during the course of the study according to food consumption and body weights.

Administration of the test diet to F_1 rats began when the rats were approximately 40 days old. After 110 days on test males and females were allowed to mate. Cohabitation consisted of a maximum of two 5-day breeding periods separated by a 7 day rest period. New pairs were introduced after the first mating. During cohabitation, males received test diet with concentration appropriate for the female partner (i.e., test diet was adjusted for the weight of the female).

F_1 litters were weaned at 21 days of age, at which time the F_0 generation was sacrificed. Thirty male and thirty females animals from each dose group were randomly chosen as parents for next generation. After weaning at 21 days, animals received test diet for 120 days and were then mated using the same protocol as for the previous generation. After weaning of the F_2 generation, all F_1 parental animals and F_2 weanlings were sacrificed.

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Litters were randomly culled to 8 pups on day 4 post-partum. All culled pups were sacrificed.

Each animal on test was observed daily for "signs of toxicity or changes in demeanor". All rats dying and moribund sacrifices were examined grossly, further pathological examination was apparently not conducted with the exception of pups with external abnormalities . Body weights and food consumption were recorded weekly with the exception of pups and lactating females. On days 1, 4, 7, 14 and 21 after birth, the number of live pups, litter weights and lactating female weights were recorded. On the day of birth, litter size and the number of live and dead pups were recorded. External abnormalities of pups were noted and these pups were necropsied. Individual weights were determined for each pup on day 21.

Statistical analysis was conducted by the laboratory using appropriate procedures. These procedures are described on pages 14 and 15 of the study report. The study report stated the following with regard to statistical evaluation:

"Body weights and food consumption were evaluated by Bartlett's test for equality of variances. Based upon the outcome of Bartlett's test, a parametric or nonparametric analysis of variance (ANOVA) was conducted. If the ANOVA was significant, Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction (Steel and Torrie, 1960) was carried out. Statistical outliers were determined for body weights and food consumption using the method of Grubbs (1969), but were not routinely excluded from further analysis. The fertility index was analyzed by the Fisher exact probability test (Siegel, 1956). Evaluation of the neonatal sex ratio on day 21 was done using the binomial distribution test as described by Steel and Torrie (1960). Survival indices and other incidence data among neonates were analyzed using the litter as the experimental unit by the Wilcoxon test as modified by Haseman and Hoel (1974) and incorporating Bonferroni's correction for pairwise comparisons.

The nominal alpha levels used for statistical evaluation included:

Bartlett's Test for Variances	= 0.01
Analysis of Variance	= 0.05
Dunnett's Test	= 0.05, two sided
Wilcoxon Rank-Sum Test	= 0.05, two-sided with Bonferroni correction (Miller, 1966)
Outlier Determination	= 0.02, two-sided = 0.01, one-sided
Fisher's Test	= 0.05, one-sided
Modified Wilcoxin Test	= 0.05, one-sided

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (i.e., type I errors) would be much greater than the above-cited alpha levels would suggest. Consequently, the final interpretation of numerical data generated in this study considered statistical analyses along with other factors such as dose-response relationships and whether the results were significant in light of other biological and pathological findings."

Results:

Three control male animals, two control female animals, one 0.5 mg/kg/day female and one 1.2 mg/kg/day male either were spontaneous deaths or moribund sacrifices in the F_0 generation. One control female died during the F_1 generation. No clinical observations during the course of the study were considered by this reviewer to have been related to test compound administration.

Mean body weights of all dosed F_0 groups were similar to corresponding control values. Food consumption values for all dosed F_0 groups were similar to corresponding control values. The few F_0 parental animals that were grossly necropsied showed no signs of treatment related pathology. Lactation body weights of dams receiving test compound were similar to control animals.

Reproductive parameters of the F_0 generation also did not appear to be affected by treatment. These parameters included the following: "fertility indices, gestation days, gestation indices, gestation survival indices, total number of live pups per litter on day 1 of lactation, cumulative pup survival indices on days 1, 4, 7, 14 and 21 of lactation, sex ratios of pups at day 21 and incidence of external alterations of F_1 pups between birth and 21 days of age". The only external abnormalities of pups that were observed were missing tails (observed in one control and one 1.2 mg/kg/day animal).

With respect to neonatal survival, the percentage of liveborn pups that survived over the course of 21 days was much higher than in previous studies and the survival rates were similar for each dose level in the F_1 generation (97.3, 96.7, 100 and 98.5% survival of liveborn pups over 21 days for the 0, 0.5, 0.8 and 1.2 mg/kg/day dose levels, respectively).

Body weights of the F_1 male rats appear to have been slightly decreased as a result of test compound administration. Total mean weight gains from day 14 after birth to day 182 on test (189 days of exposure) were 487, 435, 433 and 414 grams for the control, 0.5, 0.8 and 1.2 mg/kg/day dose levels. Significant ($p < .05$) differences were observed between control and high dose level animals for the last 4 weighing intervals. Two significantly decreased intervals were observed for the 0.8 mg/kg/day level compared to control (at day 127 and day 182). Sporadic significant decreases in the food consumption of treated males were observed compared to control animals (3 significant decreases in the 0.5 and 0.8 mg/kg/day dose levels and 5 significant decreases at 1.2 mg/kg/day). F_1 female body weights and food consumption did not appear to be effected by test compound administration.

Reproductive parameters of the F_1 generation did not appear to be effected by treatment. Reproductive parameters examined were those previously noted in this review for the F_0 generation. One pup (at 0.8 mg/kg/day) was diagnosed as being hydrocephalic and a second pup (at 1.2 mg/kg/day) was found not to have a tail. The small number of animals with external abnormalities precludes associating these findings with treatment.

Findings with respect to neonatal survival were similar to those of the previous generation. Survival at all dose levels was excellent and an effect of test compound administration on neonatal survival was not observed. Survival of liveborn pups over 21 days was 98.2, 99.5, 100 and 99.5% for the 0, 0.5, 0.8 and 1.2 mg/kg/day dose levels respectively.

Core Classification:

Although not meeting core requirements for a reproduction study (primarily due to a limited gross and no histological examination), the study is adequate to establish that the NOEL for neonatal survival is 1.2 mg/kg/day (HDT). This was the primary purpose of the study. The NOEL for other reproductive parameters is also 1.2 mg/kg/day and the NOEL for general toxicity is 0.8 mg/kg/day based on decreased weight gain observed in the 1.2 mg/kg/day male dose level. In combination with the previous reproduction study, this study is adequate to meet the requirement for a Core-Minimum Reproduction Study.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003822

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

TO: Jay Ellenberger
Registration Division (TS-767)

FROM: Gary J. Burin, Toxicologist 5/15/84
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

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 413 and 367 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
21	.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.

~~DRAFT~~

003822

MEMORANDUM

SUBJECT: Attached Data Evaluation Record, Chlorpyrifos: Two-Year Dietary Exposure Studies in Beagle Dogs

TO: Gary J. Burin, Toxicologist
SIS
Hazard Evaluation Division (TS-769)

FROM: John A. Quest, Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769)

I was asked to review the attached dog study on Chlorpyrifos for use in a registration standard. The original draft was performed by Dynamac. However, for purposes of expediency I completed the review myself and therefore the document does not have the normal DER cover page.

DATA EVALUATION RECO

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STUDY TYPE: Two-year dietary feeding studies in beagle dogs.

CITATION: McCollister SB, Kociba RJ, Gehring PJ, and Humiston CG. 1971. Results of two-year dietary feeding studies on Dowco® 179 in Beagle dogs: T35, 12-44793-18. (An unpublished report prepared by Dow Chemical, USA, Midland, MI).

ACCESSION NUMBER: 240192009.

MRID NUMBER: 00064933.

LABORATORY: Chemical Biology Research, Dow Chemical, USA.

TEST MATERIAL: Chlorpyrifos [0,0-Diethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothicate]; Dowco® 179, Lot No. CP 523-CD 235C; Purity: 98.8 percent (U.V.), 97.2 percent (GLC).

PROTOCOL: The study was conducted in two phases: Phase I in which the test compound was given for 1 year and then three months were allowed for recovery; Phase II in which compound feeding continued for 2 years.

PHASE I.

1. Eleven-month-old Beagle dogs were used.

2. Five groups (3 animals/sex/group) were fed Dowco® 179 in the diet at dosage levels of 0.01, 0.03, 0.1, 1.0 or 3.0 mg/kg/d for one year. A sixth group of animals served as controls. The composition of the control diet was not specified in the sponsors report.

3. The following parameters were evaluated: apearance and signs of increased cholinergic activity (daily); body weights in the one-year study were reported only at the end of the study; food consumption (weekly during months 1-3, and one week of each month thereafter), hematology* (prior to feeding and at 1, 2, 3, and 12 months on control animals and those which received 1.0 and 3.0 mg/kg/day); urinalysis* (pretest, at one month, and prior to sacrifice on control animals and those which received 1.0 and 3.0 mg/kg/day); plasma and RBC's cholinesterase activity (pretest, 1 and 2 weeks, and 1, 3, 6, 9, and 12 months on test in all dogs, six weeks after recovery on control and 0.1, 1.0 and 3.0 mg/kg/day groups, 3 months after recovery on control and 1.0 and 3.0 mg/kg/day groups);

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*Parameters studied included packed cell volume, hemoglobin, RBC count, WBC total and differential count, and prothrombin time.

brain cholinesterase activity (one dog/sex from each group at the end of the one year test period, and two dogs/sex from the control group and the 1.0 and 3.0 mg/kg/day groups at the end of 3 month recovery period); and other clinical chemistry studies ** (all dogs, pretest and at 1, 3, 6, and 12 months).

4. At the end of the one year period, one male and one female dog from each group were necropsied. The weights of heart, liver, kidneys, spleen, testes, and brain were determined.

5. Histopathological examination of 29 tissues from each animal in the control group, and groups that received 1.0 and 3.0 mg/kg/day was performed. The tissues examined were those required by the EPA Guidelines (1982) except for skin, thymus, salivary glands, caecum, rectum and gall bladder. All tissues were preserved in 10% formalin.

6. Students' "t" test was used to test for the significance of changes in cholinesterase activity.

Phase II.

1. Ten-month-old Beagle dogs were used.

2. Five groups (4 animals/sex/group) were fed Dowco® 179 in diet at dosage levels of 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day for two years; a sixth group of animals served as control. The composition of the control diet was not specified in the sponsor's report.

3. Body weights in the two-year study were reported at 6, 12, 18, and 24 months and not at weekly or biweekly intervals as stated in the protocol. Appearance of the animals, and food consumption were noted as in Phase I. The following parameters were evaluated in all animals in the control group and those which received 1.0 and 3.0 mg/kg/day: hematology*** (pretest, and at 1, 6, 12, 18, and 24 months); urinalysis*** (pretest, and at 1, 12, and 24 months); plasma and RBC's cholinesterase activity (pretest, at 1 week, and at 1, 3, 6, 12, 15, 18, and 24 months); brain cholinesterase activity (at 24 months); and other clinical chemistry studies*** (pretest and after 1 and 24 months in all dogs, and also in dogs in the 0, 1 and 3 mg/kg dose groups after 6, 12 and 18 months). Bromsulfalein retention was measured in all dogs (pretest and terminally), and on control dogs and those which received 1.0 and 3.0 mg/kg/day (at 12 months). All dogs were given complete physical exams including neurologic and ophthalmoscopic examination prior to termination.

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*Parameters studied included specific gravity, pH, sugar and albumen content, and microscopic examination of the sediment.

**Parameters included BUN, AP, SGOT, and SGPT.

***Parameters studied were the same as in Phase I.

4. All animals were sacrificed and necropsied at the end of two years and organ weights of the brain, heart, liver, kidneys, spleen, and testes were determined.

5. Histopathologic examination was performed on 29 tissues from each animal in the control group and those which received 3.0 mg/kg/day. The tissues examined and preserved were those specified for Phase I of the study.

RESULTS: The results obtained in this study were similar in Phase I and II; any differences observed between the two phases of the study are indicated below.

1. Appearance and Behavior: No data were presented. However, the authors stated that "no clinical signs of toxicity" and no "greater than normal cholinergic activity" were observed.

2. Body Weights: Male dogs that received the test compound for one year showed a slight increase in body weight gain (5-10 percent) over those of the control (4 percent). Females that received 0.03 mg/kg/day, showed a 4 percent body weight gain as compared to 9 percent in the control.

   Dogs which were used for the two-year study, showed an inconsistent gain in body weights. The percent increase in their mean body weights as compared to the initial mean body weight is shown in Table 1.

   TABLE 1. Body Weight Gain (Percent of Initial Body Weight) at the end of the 2-Year Study

Sex	Control	Dose (mg/kg/day) 0.01	0.03	0.1	1.0	3.1
Males	14	23	18	13	11	-
Females	25	17	19	25	14	19

   The smaller than control body weight gain of the males which received 3.0 mg/kg/d was also observed at 12 and 13 months.

3. Food Consumption: In the one-year study, cumulative mean food consumption for the 12 months was increased in males which received 0.1 and 1.0 mg/kg/day; the increase averaged 9.1 and 11.2 percent, respectively. Females in the two highest dose groups (1.0 and 3.0 mg/kg/day) showed a decrease in the same parameter averaged 12.6 percent and 9.4 percent of the control values, respectively. However, in both sexes, there was no dose-effect relationship.

No dose-related change in the cumulative mean food consumption of males in the two-year study was observed. Females of the smallest (0.01 mg/kg/d) and the highest (3.0 mg/kg/d) dose groups showed a decrease of 11.6 percent, and an increase of 13.6 percent in the cumulative mean food consumption, respectively.

Hematology and Urinalysis: No compound-related effects were found in the various hematologic parameters tested and in the urinalyses performed both one- and two-year studies.

Cholinesterase Activity:

°Plasma ChE activity, in the one-year study, was significantly decreased in all male and female dogs except the 0.01 mg/kg/day group. The effect was dose-dependent and appeared as early as 7 days in the treatment. The percentage decrease from control in plasma ChE activity averaged 11.2, 25.9, 45.1, 65.8 and 74.6 percent in male dogs receiving 0.01, 0.03, 0.1, 1.0 and 3.0 mg/kg/d of Dowco® 179, respectively. The decrease in the plasma ChE activity of females averaged 5.6, 20.9, 38.8, 63.8 and 71.0 percent of the control values at the above dosage levels, respectively. Cholinesterase activity returned to normal levels 14 days after cessation of test-chemical administration. The decrease in plasma ChE activity induced by the 0.1, 1.0 and 3.0 mg/kg/d dose levels in both male and female animals, was statistically significant at all test intervals; the decrease in the same parameter induced by the 0.03 mg/kg/d dose level was statistically significant at certain test intervals only; and the decrease observed at the 0.01 mg/kg/day dose level was not statistically significant. The NOEL was 0.01 mg/kg/day for male and female dogs.

Plasma cholinesterase activity in the 2-year study followed a similar pattern. In males, doses of 0.1, 1, and 3 mg/kg produced significant decreases in ChE activity (-40, -64 and -75%, respectively ; the 0.03 mg/kg dose reduced ChE activity -19%; this was not a statistically significant effect but it may be of biological significance. In females, doses of 0.1, 1, and 3 mg/kg produced significant decreases in ChE activity (-30, -59, and -63%, respectively) whereas 0.03 mg/kg did not (-0.3%). The NOEL was 0.01 mg/kg for male and 0.03 mg/kg female dogs.

Red blood cell (RBC) ChE activity was significantly reduced only in animals that received 1 and 3 mg/kg for 1 year, males: (-51 and -66%, respectively; females: -49 and -71%, respectively). No significant changes occurred at doses of 0.01-0.1 mg/kg. RBC ChE activity returned to normal after 90 days in animals which received the 1 and 3 mg/kg doses. The NOEL was 0.1 mg/kg for males and females.

all F_1 parental animals and F_2 weanlings were sacrificed. 27

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In the two-year study RBC ChE activity was also significantly decreased in male and female animals that received the 1.0 and 3.0 mg/kg/day dose levels (males: -54 and -73%, respectively; females: -54 and -71%, respectively); 0.01 to 0.1 dosage levels showed no significant effect. The NOEL was 0.1 mg/kg/day for males and females.

°Brain cholinesterase activity of animals maintained on Dowco® 179 for one year was not markedly changed from the control group. However, only one animal/sex/group was used to determine brain ChE activity. Animals (2/sex/group) that received 1.0 and 3.0 mg/kg/day and which were sacrificed 3 months after recovery, however, showed a slight but not dose-related decrease in brain ChE activity.

In the two-year study, the decrease in brain ChE activity with reference to control levels averaged 1.5, 6.7, 8.3, 7.2, and 20.8% in 4 male dogs per group receiving 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day of the test material, respectively. In the same study, the percent change from control in brain ChE were -7.1, -2.8, +11.8, +5.8, and -19.4 in 4 female dogs per group receiving respectively the same doses as the males. These changes were not statistically significant.

6. Other Clinical Chemistry Studies: A slight increase in alkaline phosphatase, and a slight decrease in SGOT activity was noticed in animals that received 1.0 and 3.0 mg/kg/day Dowco 179 for 1 year. However, these changes were neither dose-related nor statistically significant. In the two-year study, changes in these parameters were minimal. At 6, 12, and 18 months the clinical chemistry parameters studies (AP, SGOT, SGPT, BSP) were evaluated only in groups that received 1.0 and 3.0 mg/kg/d dosage levels, no test-compound-related effect was observed.

7. Organ Weights: No dose-related changes were observed in the weights of heart, liver, kidneys, spleen, testes, or brain, nor in the organ weight/body ratio for animals sacrificed at the end of 1 year, or after a 3-month recovery period. However, only one animal/sex/group was sacrificed at the end of the 1 year period, the remaining two animals sex/group were sacrificed after a 3-month recovery period.

Organ weights and organ body weight ratios for animals sacrificed at the end of the two-year period showed changes which were not dose-related. However, although not dose-related, a generalized increase in the heart and liver weights of male animals at all dose levels were observed. The most noticeable change from control was an increase in both the average liver weight, and in the liver/body weight ratios for males that received 3 mg/kg/day of Dowco® 179. The male liver/body weight ratios were 2.4 for the control group and 3.47 for the highest dose group. These changes were statistically significant ($p < 0.05$).

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8. Gross Pathology: Both control and treated groups showed similar results on gross pathological examination.

9. Microscopic Examination: One dog from the 3 mg/kg/d group in the one-year study had subcutaneous histocytoma diagnosed from a biopsy during the first month of study. One dog that received 1 mg/kg/day showed an accumulation of inflammatory cells in renal tubules.

In the two-year study, only tissues from control dogs and those that received 3.0 mg/kg/day were examined microscopically. Of the four males in the dosed group, two animals showed pleocellular foci in the liver and two showed mineralized foci in renal medullary tubules. These same lesions, at similar incidences, were found in the 3.0 mg/kg/d dosed females and in control dogs of both sexes.

DISCUSSION:

Plasma and RBC cholinesterase activities were significantly decreased during the study in animals at dosages as low as 0.03 mg/kg/day. This effect appeared as early as 7 days in the study and was reversed in the plasma as early as 14 days in the recovery period. Only animals at the highest dose level showed apparent inhibition of brain cholinesterase activity. The overall NOEL for ChE activity was 0.01 mg/kg/day for male and female dogs.

The increase in liver weight and in liver/body weight ratios for male dogs that received 3 mg/kg/day of Dowco® 179 was not associated with histopathological changes. It seems that the liver is the target organ for Dowco® 179 toxicity, as demonstrated by the significant increase in male liver weight at 3 mg/kg/day. No other toxic effects than ChE inhibition and liver organ weight increase were demonstrated to be due to the administration of the test compound.

Dogs used in both phases of this study were eleven (Phase I) and ten (Phase II) months old at the initiation of the study, which exceeds the usual range of 4-6 months used in this type of study. Furthermore, discrepancies existed between the protocol and the actual study. For instance, while the protocol specified that body weights would be determined at a weekly interval during the first six months and biweekly thereafter, data presented included body weights at 12 months in the one-year study, and at 6, 12, 18, and 24 months in the two year study. Similar discrepancies were observed in food consumption, clinical chemistry and histopathology parameters.

Microscopic examination tissues was not performed on any female nor on 3 out of the 4 males that received 1 mg/kg/day, contrary to specifications in the protocol. Furthermore, one dog was diagnosed to have had subcutaneous histocytoma as early as one month in the study and should have been excluded from the study.

CONCLUSIONS

Under the conditions of this study, dietary administration of Dowco® 179 to Beagle dogs for two years induced the following effects: 1) an inhibition of plasma and RBC cholinesterase activity; 2) an increase of liver and liver/body weight ratios in male animals which received 3 mg/kg/day, the highest dose administered. The overall NOEL for Dowco® in this study is considered to be 0.01 mg/kg/day based on the inhibition of plasma cholinesterase in both sexes of dogs.

CORE CLASSIFICATION: Supplementary.

This classification is based on the following deficiencies:

1. Data was not available at the intervals specified in the protocol for several of the parameters that were monitored (e.g. body weight, ophthalmology, etc.).

2. Histology data was not presented for each of the individual tissues examined.

The classification may be upgraded if the deficiencies can be adequately corrected.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-01-6561
MRID 00158823
April 6, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS (Dowco 179)

2-Year Chronic Toxicity/Oncogenicity Feeding Study—Rats

CITATION: McCollister, S.B., Kociba, R.J., Gehring, P.J., and Humiston, C.G. Results of two-year dietary feeding studies on Dowco® 179 in rats. Unpublished study by Dow Chemical Co. dated September 20, 1971.

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DATA EVALUATION RECORD 003822

STUDY TYPE: 2-year chronic toxicity/oncogenicity feeding study—rats.

CITATION: McCollister, S.B., Kociba, R.J., Gehring, P.J., and Humiston, C.G. Results of two-year dietary feeding studies on Dowco^{R} 179 in rats. Unpublished study by Dow Chemical Co. dated September 20, 1971.

ACCESSION NUMBER: Not available.

MRID NUMBER: 00081270.

LABORATORY: Chemical Biology Research, Dow Chemical USA, Midland Mich.

TEST MATERIAL: Chlorpyrifos [0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl)-phosphorothioate], Dowco^{R} 179, was from Lot No. CP523-CD235C and assayed 98.8 percent pure by ultraviolet spectrum and 97.2 percent pure by gas liquid chromatography.

PROTOCOL:

1. Sherman rats, source unspecified, were used in the study. Primary groups of 25/sex/dose level (300 total) were maintained in the study for 2 years and supplementary groups (57/sex/dose level, total 684) were used for interim pathologic examinations and periodic cholinesterase determinations as shown in Table 1. At initiation of dosing the animals were 7 weeks old, and males weighed approximately 165 g and females approximately 135 g. Chlorpyrifos was given in the diet at concentrations which resulted in dosages to the test animals of 0 (control), 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day. The animals were housed 2/cage in wire mesh cages for 3 months and were individually caged thereafter. Food and water were available ad libitum.

2. An appropriate amount of test compound dissolved in acetone was mixed with ground Purina Lab Chow to prepare a 1 percent premix. Test diets were prepared by mixing an appropriate amount of premix and ground feed. The concentration of chemical in diets was adjusted weekly for the first 3 months and as often as necessary thereafter to maintain the designated dosages (mg/kg/day). Diets were prepared weekly or biweekly. Periodic analysis by a gas liquid chromatography method was used to check the dietary levels of test compound. The test material was stable in feed at room temperature for one week. After 7 weeks storage, recovery was 87-92 percent.

TABLE 1. Supplementary Groups of Animals

Purpose	No./group/sex
1 week ChE[a] determination	5
1 month ChE determination	5
3 month ChE determination	5
6 month ChE determination	5
9 month ChE determination	5
12 month ChE determination	6
12 month necropsy	5
12 month plus recovery[b] necropsy and ChE	7
18 month ChE determination	7
18 month necropsy	7

[a] ChE, cholinesterase.

[b] Fed dosed diet for 12 months and then control diet for 50 days.

3. Animals were observed "frequently" [sic] for mortality, moribundity and for toxic signs, especially for evidence of cholinergic responses. Body weights were recorded twice weekly for the first month, weekly during months 2-4 and bi-weekly thereafter. Food consumption for the primary groups was recorded weekly for the first 3 months and one week/month thereafter.

4. Hematologic studies were performed on 5 rats/sex of the 0, 1.0 and 3.0 mg/kg groups at 1, 6, 12, 18, and 24 months, except at 12 months 10 females of each of these groups were examined. The parameters measured were hematocrit, hemoglobin, erythrocyte count, and total and differential leukocyte count. Urinalyses were conducted on 5 rats/sex from the above groups at the same time intervals.

5. Blood urea nitrogen (BUN), alkaline phosphatase (AP), and serum glutamic pyruvic transaminase were measured on rats sacrificed at 12 months (5/sex/group), 18 months (7/sex/group), and 24 months (all males and 4-5 females/group).

6. Cholinesterase activity of plasma and erythrocytes was measured on 5-7 rats/sex/ dosage group (supplementary groups) at 1 week, and at 1, 3, 6, 9, 12, and 18 months and on all surviving males and all but 4-5 females/group at the 24 month sacrifice. Brain cholinesterase was asured at 6, 12, and 18 months. In addition, blood and brain cholinesterase activity were measured in a recovery group of 7 rats/sex/dose, maintained on test diets for 12 months and then fed control diets for 7-8 weeks.

7. Animals were sacrificed and necropsies conducted at the following intervals: 12 months, 5 rats/sex/group; 18 months, 7 rats/sex/group; and 24 months, all surviving rats. Brain, heart, liver, kidneys, spleen and testes from each animal were weighed. Portions of the above organs and the following tissues were preserved in formalin: eyes, pituitary, thyroid, parathyroid, trachea, esophagus, lungs, aorta, stomach, pancreas, small intestine, colon, mesenteric lymph nodes, urinary bladder, accessory sex glands, ovaries, uterus, skeletal muscle, sciatic nerve, sternum and bone marrow, adrenals, and any nodule or mass.

8. The preserved tissues were examined microscopically for animals in the 0, 1.0 and 3.0 mg/kg groups at 12 month sacrifice, and for animals in the 0 and 3.0 mg/kg groups at recovery sacrifice, 18 month sacrifice, and those sacrificed at termination of the study. In addition, tissues from all test groups and controls were examined for animals that died during the study.

10. Hematologic data, clinical chemistry data, cholinesterase activity data, final body weight, organ weight and organ/body weight ratio data were analyzed by Student's t-test for significant differences between control and test mean values.

RESULTS:

Observations: It was stated that there were no changes in appearance, signs of toxicity, or cholinergic responses in any animals at any time in the study; however, no data were available to support this.

Mortality: Data on mortality are summarized in Table 2. There was no compound-related effect on mortality or on the times at which deaths occurred. Survival at 18 months ranged from 64 to 92 percent in all groups.

TABLE 2. Mortality Data[a]

Group	Dose (mg/kg/day)					
	0	0.01	0.03	0.1	1.0	3.0
Males						
No. of deaths (24 mos.)	15	16	19	18	12	15
18-month survival (percent)	68	68	64	80	80	64
Females						
No. of deaths (24 mos)	14	11	11	12	8	15
18 month survival (percent)	80	76	92	92	88	92

[a]In groups of 25 rats/sex started on test.

Body Weights and Food Consumption: The mean body weights of males and females administered test compound were similar to or greater than controls throughout the two-year study. However, mean body weights were presented graphically in the report, means and standard deviations were not tabulated, individual animal data were not present, and statistical analysis was not available. Mean food consumption was similar in all groups of males and females throughout the study.

Hematology: Although there were scattered significant differences in hemoglobin values and white cell counts between control and test groups of rats, there were no time- or dose-related effects and the values were within the normal range. There was no compound-related effect on any other hematologic parameter.

Cholinesterase Activity: Doses up to 0.1 mg/kg/day of chlorpyrifos caused no significant depression of cholinesterase activity of plasma, erythrocyte, or brain when compared to controls. However, there were dose-related depressions of plasma and erythrocyte cholinesterase activity throughout the study in male and female animals at 1.0 and 3.0 mg/kg/day test compound when compared with controls (Tables 3 and 4). The levels of plasma cholinesterase activity were somewhat more depressed in females

TABLE 3. Mean Cholinesterase Activity of Plasma as Percent of Control Value

Days on Diets	Dose Level (mg/kg/day)					
	Males			Females		
	Control	1.0	3.0	Control	1.0	3.0
7	100	86.7	59.0*	100	66.5	44.7
30	100	94.1	79.3	100	55.8*	33.0*
90	100	97.1	70.5*	100	46.6*	39.3*
180	100	82.4	80.2	100	38.1*	27.3*
279	100	61.5*	61.5*	100	31.0*	26.0*
365	100	80.3	66.4*	100	49.5*	36.1*
365 + 50 recovery[a]	100	83.4*	81.8	100	98.7	92.0
547	100	66.2*	61.5*	100	47.8*	34.0*
730	100	73.9*	67.5*	100	43.7*	31.8*

[a]50 days recovery on control diet after 365 days of dosing.

*Statistically different from control at $p<0.05$ when absolute values were compared by the Student t-test.

TABLE 4. Mean Cholinesterase Activity of Erythrocytes as Percent of Control Value

Days on Diets	Dose Level (mg/kg/day)					
	Males			Females		
	Control	1.0	3.0	Control	1.0	3.0
7	100	86.7	40.5*	100	88.2	32.1*
30	100	31.1*	4.9*	100	34.3*	4.8*
90	100	42.2*	35.9*	100	42.2*	23.9*
180	100	15.9*	9.4*	100	22.0*	12.2*
279	100	15.8*	11.3*	100	24.2*	10.0*
365	100	9.5*	1.4*	100	18.5*	4.1*
365 + 50 recovery[a]	100	91.1	105.3	100	94.6	104.6
547	100	16.0*	11.5*	100	15.8*	11.5*
730	100	25.9*	16.4*	100	32.8*	18.4*

[a]50 days recovery on control diet after 365 days of dosing.

*Statistically different from control at $p<0.05$ when absolute values were compared by the Student t-test.

than in males at 1.0 and 3.0 mg/kg/day. When rats were fed test compound in the diets at 1.0 or 3.0 mg/kg/day for one year and then fed control diets for a 7-week recovery period, cholinesterase activity of plasma and erythrocytes returned to normal.

Brain cholinesterase activity was depressed at all sampling times in both males and females at 3.0 mg/kg/day and was marginally depressed at 1.0 mg/kg/day when compared to controls. Animals fed the test compound for a year at 3.0 mg/kg/day and then control diets for a 7-week period were considered to have brain normal cholinesterase activity (Table 5) on the bases of the expected biological variation of cholinesterase activity and the variation of the methodology used to determine this activity.

TABLE 5. Mean Cholinesterase Activity of Brain as Percent of Control Value

Days on Diets	Dose Level (mg/kg/day)					
	Males			Females		
	Control	1.0	3.0	Control	1.0	3.0
180	100	97.3	46.9*	100	90.7*	61.4*
365	100	88.0*	45.2*	100	92.6	47.4*
365 + 50 recovery[a]	100	98.0	92.9*	100	98.2	90.3
547	100	90.3*	70.5*	100	92.1*	62.6*
730	100	84.2*	60.4*	100	95.1	57.5*

[a]50 days recovery on control diet after 365 days of dosing.

*Statistically different from control at $p<0.05$ when absolute values were compared by the Student t-test.

Other Clinical Chemistry: There were no compound-related changes in blood urea nitrogen, alkaline phosphatase, or serum glutamic pyruvic transaminase.

Urinalysis: There were no compound-related effects on the urinary parameters determined.

Organ Weights: At the one year sacrifice there were sporadic increases in heart, liver, and kidney weights and organ/body weight ratios in dosed females compared to controls, but the increases were not dose-related nor were they considered toxicologically significant. At the eighteen month sacrifice, organ weights were similar in treated and control males and females. At the two-year sacrifice there were slight but significant increases in the weights of kidney, liver, and spleen in males at the 3.0 mg/kg/day dose compared to controls, but the organ to body weight ratios were not significantly increased when compared to controls.

Gross Necropsy: Some gross lesions were noted, but there was no increased incidence of any lesion in any dosed group of males or females when compared with controls. There was a tabulation of gross alterations for individual animals that died during the study. For animals sacrificed at termination of the study, individual gross findings were not presented, but summary tables of lesions indicated that tissues from all animals were grossly examined. Summary tables of gross lesions (but no individual data) were also present for animals at interim sacrifices.

Histopathology: For animals that died or were sacrificed moribund during the study, there was a tabulation of histopathologic alterations for individual animals. For animals at interim sacrifices and those sacrificed at study termination, individual histopathologic data were not presented.

A summary of incidence of non-neoplastic lesions for animals at interim sacrifices is presented in Table 6. Summary data was present for control and high dose animals; in addition, summary data was present for the 1.0 mg/kg/day group at the 12 month sacrifice. No compound-related effects were noted.

Table 7 summaries non-neoplastic lesions in animals in the control and 3.0 mg/kg/day groups that died or were sacrificed at study termination. This tabulation utilized individual data for animals that died and summary data for animals that were sacrificed. There was no increased incidence in lesions in the 3.0 mg/kg/day group of males or females when compared to controls. Table 8 summarizes the number of animals in the control and 3.0 mg/kg/day groups with neoplastic lesions. Individual data were only available for animals that died. There was no increased incidence of any tumor in males or females at 3.0 mg/kg/day when compared with controls. The report listed the tumors in animals in lower dose groups but this data could not be validated because it was based on summary data and only tissues with grossly observed masses were examined histologically.

DISCUSSION:

Insufficient numbers of animals were employed to satisfy core guideline data for oncogenicity testing but adequate numbers were used to satisfy core minimum data for oncogenicity. Adequate numbers of animals were utilized for chronic toxicity testing; greater than 50 percent survived 18 months and greater than 25 percent survived 24 months.

The histopathology data was limited, since data for individual animals was only available for animals that died or were sacrificed moribund; only summary data was present for animals sacrificed at termination of the stucy.

The study was further limited since there were no available data on clinical observations or individual body weight data. Hematology data was limited since only at 12 months were 10 animals/sex in the control and high dose groups examined; at other intervals, 5 animals/sex/dose were examined. Urinalyses were performed at the usual intervals in the control, 1.0 and 3.0 mg/kg/day groups but only 5 animals/sex/dose were

TABLE 6. Incidence of Non Neoplastic Lesions at Interim Sacrifices

Organ/Lesion	Group (mg/kg/day)													
	12 Mos. Sacrifice						Recovery Sacrifice				18 Mos. Sacrifice			
	Males			Females			Males		Females		Males		Females	
	0	1.0	3.0	0	1.0	3.0	0	3.0	0	3.0	0	3.0	0	3.0
Lungs,														
chronic pneumonia	4/4	5/5	4/5	5/5	4/5	5/5	4/7	5/6	4/7	6/6	6/6	5/5	4/7	6/6
minimal	–	–	–	–	–	–	2/7	1/6	3/7	–	–	–	2/7	–
moderate to severe	–	–	–	–	–	–	–	–	–	–	–	–	–	–
submucosal tracheitis	1/4	3/5	–	4/5	4/4	5/5	–	–	–	–	–	–	–	–
Liver,														
mononuclear cell foci	1/4	1/5	–	3/5	3/4	5/5	3/7	6/6	4/7	4/6	–	–	1/7	–
Kidney,														
glomerular alterations	2/4	3/5	4/5	4/5	3/4	5/5	–	–	–	–	–	–	–	–
cast formation	2/4	3/5	4/5	4/5	3/4	4/5	–	–	–	–	–	–	–	–
minimal chron. nephritis	–	–	–	–	–	–	2/7	4/6	5/7	5/6	4/6	3/5	3/7	4/6
mod. to severe nephritis	–	–	–	–	–	–	5/7	–	–	1/6	1/6	2/5	3/7	1/6
dilation of renal tubules	3/4	2/5	4/5	2/5	1/4	2/5	–	–	–	–	–	–	–	–

[a]The data are expressed as: number of occurrences/number of animals examined histologically.

TABLE 7. Number of Animals with Non-Neoplastic Histopathologic Lesions

Organ/Lesion	Dose Level (mg/kg/day)			
	Males		Females	
	0	3.0	0	3.0
No. of animals examined	24	25	25	25
Lung				
chronic murine pneumonia	22	18	24	16
Liver				
mononuclear cell foci	4	1	7	2
pleocellular foci	-	-	2	1
Kidney				
dilation of renal pelvis	-	1	-	-
chronic nephritis:				
-severe	2	3	5	3
-minimum to moderate	14	9	14	13
Heart				
myocardial degeneration	11	9	8	12
mesenteric periarteritis	-	2	-	3
Adrenal				
hematocyst	1	2	7	7
Testes				
atrophy	1	2		
Stomach				
focal gastritis	-	1	-	1
Spleen				
hyperplasia	1	-	1	2
cellular depletion	1	-	-	-
extramedullary hemopoiesis	2	1	2	2

[a]Prepared by this reviewer using individual histopathology data for animals that died during the study and summary incidence data for animals sacrificed at 24 months.

TABLE 8. Number of Animals with Neoplastic Histopathologic Lesions

Organ/Lesion	Dose Level (mg/kg/day)			
	Males		Females	
	0	3.0	0	3.0
Malignant lymphoma	2	0	0	1
Skin, fibrosarcoma	1	0	0	0
Skin, subcutaneous sarcoma	0	1	0	0
Thyroid, carcinoma	0	1	2	0
Thyroid adenoma	0	0	1	0
Adrenal, pheochromocytoma	1	1	0	0
Pituitary, adenoma	0	0	5	6
Pancreas, adenoma	0	0	1	0
Uterus, adenoma	-	-	1	0
Uterus, polyps	-	-	2	0
Uterus, fibropapilloma	-	-	1	0
Mammary, fibroadenoma	-	-	2	2
No. of animals examined	24	25	25	25

[a]Prepared by this reviewer using individual histopathology data for animals that died during the study and summary incidence data for animals sacrificed at 24 months.

studied. Clinical chemistry determinations were made on 10 animals group only for males at 24 months. For males at other intervals and for females at all intervals less than 10 animals/group had blood chemistry determinations.

Although there were no cholinergic signs noted even at the highest dose, there was severe inhibition of erythrocytes cholinesterase (96-98 percent) at the one year interval in animals at 3.0 mg/kg/day, and approximately a 50 percent inhibition of brain cholinesterase at this level and interval when compared with controls. This would indicate that a maximum tolerated dose was probably used. However, no other compound-related toxicity was noted.

Since only summary data for gross findings and histopathology were present for interim sacrificed and study termination sacrificed animals, the study as reported is considered as seriously limited. If the registrant can supply data on clinical observations, individual animal data on body weights, and individual animal gross and histopathologic observations on all required tissues, the core classification may be reconsidered.

CONCLUSIONS:

Chlorpyrifos was not oncogenic to male or female Sherman rats when fed at levels of up to and including 3.0 mg/kg/day for two years. However, individual histopathology data were not present to support this conclusion. The only compound related effects were depression of cholinesterase activity of plasma and erythrocytes at dose levels of 1.0 and 3.0 mg/kg/day and of brain at dose levels of 3.0 mg/kg/day. Inhibition of cholinesterase activity was reversible; rats maintained on diets free of chlorpyrifos for 7 weeks after a year of dosing showed recovery of cholinesterase to control levels. Based on inhibition of cholinesterase activity, a LEL of 1.0 mg/kg/day and a NOEL of 0.1 mg/kg/day can be tentatively established.

CORE CLASSIFICATION: Supplementary.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

003822

EPA: 68-01-6561
TASK: 43
April 4, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS

Subchronic Oral Toxicity

CITATION: Coulston F, Goldberg L, Abraham R, Benitz KF, Griffin TB, and Norvell M. 1971. Final report on safety evaluation and metabolic studies on Dowco 179 (IN 151). (Unpublished study received Sept. 18, 1980 under 464-557; prepared by Albany Medical College, Institute of Experimental Pathology and Toxicology, submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:099643-B).

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity - Monkeys.

CITATION: Coulston F, Goldberg L, Abraham R, Benitz KF, Griffin TB, and Norvell M. 1971. Final report on safety evaluation and metabolic studies on Dowco 179 (IN 151). (Unpublished study received Sep. 18, 1980 under 464-557; prepared by Albany Medical College, Institute of Experimental Pathology and Toxicology, submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:099643-B).

ACCESSION NUMBER: 240090306.

MRID NUMBER: 00043244.

LABORATORY: Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY, 12208.

TEST MATERIAL: The test material was identified as "Technical Dowco 179," a tradename for chlorpyrifos. The source of the test material was not specified.

PROTOCOL:

1. Fourteen Rhesus monkeys (Macaca mulatta) of unspecified age were placed into 4 groups, on the basis of body weight and treated by gavage with 0.08, 0.40, and 2.00 mg/kg/day of the test material for 6 months (Table 1). The test material was given as a suspension in 1 percent aqueous gum tragacanth; suspensions were prepared weekly.

TABLE 1. Experimental Design

Group	No. of Animals/Sex	Dosage (mg/kg/day)
1	2M, 2F	Vehicle only
2	2M, 1F	0.08
3	2M, 1F	0.40
4	2M, 2F	2.00

The animals were acclimated for 6 weeks prior to the study, housed individually in environmentally controlled quarters during the study, and provided with water and standard laboratory ration ad libitum.

2. Observations for signs of toxicity, changes in behavior, and appearance of tissue masses were made daily. Individual body weights were recorded monthly.

3. Blood samples were taken from all animals at 2, 4, and 6 months for hematologic measurements (erythrocyte and leukocyte counts, hemoglobin, and hematocrit) and clinical chemistry determinations (calcium, phosphorous, glucose, BUN, uric acid, cholesterol, bilirubin, total protein, albumin, LDH, and SGOT). Differential leukocyte counts were also made at 4 and 6 months.

4. Erythrocyte and plasma cholinesterase activities were determined for all animals at -2, -1, 1, 3, 5, 16, and 24 weeks and for one monkey from each group that was sacrificed at 3 months (12 weeks). Brain cholinesterase activity was determined on the animals which were sacrificed at 3 months and on 6 animals that were sacrificed at 5 months (see below). Also after these sacrifices, samples of liver tissue were obtained from each animal, and the activity of biphenyl hydroxylase determined in the "9,000xg fraction" from each sample. The sacrifices were accomplished by injection of a "lethal solution."

5. The remaining animals were sacrificed at the termination of the study (6 months). Of these animals, one male and one female from the control, mid-dose, and high-dose groups were examined for gross pathology. Samples of 26 tissues from these animals were preserved and examined for histopathologic changes. In addition, samples of liver and kidney tissues were taken from 2 females of the control group, from 1 female each from the low- and mid-dose groups, and from 2 males from the high-dose group and examined by electron microscopy for pathology and by histochemistry for distribution of lysosomes.

6. After 16 weeks of dosing, urine was collected from 1 male and 2 females of the low-dose group and 1 male and 1 female each from the mid-dose and high-dose group over a 24-hour period. These samples were analyzed for Dowco 179 and 3, 5, 6-trichloro-2-pyridinol (a major metabolite) by gas chromatography. These results are presented in a separate DER.

RESULTS:

Clinical Observations/Mortality: No signs of toxicity, behavioral changes, or palpable tissue masses were reported. One animal (a female in the high-dose group) was found dead after 4-1/2 months of dosing. From the results of the gross and histopathologic examinations, the authors attributed the death to a systemic infection resulting in pulmonary edema and failure of the right ventricle.

Body Weight: All monkeys gained weight at a steady rate; there were no dose-related differences among the average weight gains of the groups.

Hematology: None of the parameters showed any test material dose related changes.

Clinical Chemistry: None of the parameters showed any alterations that could be related to the test material.

Cholinesterase/Biphenyl Hydroxylase Activities: Plasma cholinesterase activity was depressed to 41 and 19 percent of control activity in the mid- and high-dose groups, respectively, after one week of dosing and remained depressed for the remainder of the study (Table 2). Erythrocyte cholinesterase activity was also depressed to 74 percent of control after 3 weeks of dosing in the mid-dose group and to 28 percent of control after 1 week of dosing in the high-dose group (Table 2). No effect on brain cholinesterase was noted at 3 or 6 months, nor was liver biphenyl hydroxylase activity affected at 3 or 6 months.

TABLE 2. Mean Plasma and Erythrocyte Cholinesterase Activity[a] in Monkeys Dosed with Dowco 179

Activity	Dose (mg/kg)	Week of Administration						
		-1	1	3	5	12[b]	16[c]	24[c]
Plasma	0.08	108	69	96	71	75	74	86
	0.40	106	41	25	26	24	28	39
	2.00	98	19	17	18	12	30	45
Erythrocyte	0.08	90	89	82	90	79	87	87
	0.40	102	85	74	71	67	68	73
	2.00	94	28	21	22	13	27	62

[a]Expressed as percent of control activity; based on the mean of 3 animals unless otherwise indicated.

[b]One animal sampled.

[c]Two animals sampled.

Gross and Histopathology: No gross or histologic effects were found that were related to dosing. The authors stated that the lesions observed were either minor in degree or were "spontaneously occurring events". Examination of liver and kidney tissue by electron microscope revealed no test material-related differences between control and dosed groups. Histochemical examination of the distribution of lysosomes in these tissues also revealed no test material-related differences.

DISCUSSION:

In this study, determination of possible effects on cholinesterase activity, hematology, clinical chemistry, and body weight gain was adequately conducted. However, a limited number of animals were examined for gross or histopathologic lesions. Only 2 control, 2 mid-dose, and 3 high-dose animals were thoroughly examined for gross and histopathologic lesions (Table 3). In addition, no rationale was provided for the selection of the animals for examination. Two minor points were noted: The method of sacrifice was not explicitly stated and the results of the determination of biphenyl hydroxylase activity in the interim sacrifice animals were not presented.

TABLE 3. Conduct of Pathologic Examinations

Group (mg/kg)	Animal No./Sex	Type of Examination[a] (Tissues Examined)
Control[b]	939 F	G, H (all tissues), E (liver, kidney)
	942 F	Not examined
	943 M	Not examined
	974 M	G, H (all tissues)
0.08	833 M	Not examined
	838 F	E (liver, kidney)
	940 M	Not examined
0.40	840 F	G, H (all tissues) E (liver, kidney)
	938 M	Not examined
	941 M	G, H (all tissues)
2.00	935 M	E (liver, kidney)
	936 M	G, H (all tissues)
	937 F	G, H (all tissues), E (liver, kidney)
	976 F[c]	G, H (all tissues)

[a]G - gross examination, H - histopathologic examination, E - electron microscopic examination.

[b]A female animal no. 969 was reported to have had samples of liver and kidney examined by electron microscope, but nowhere else in the report is this animal number used.

[c]This animal was found dead after 4-1/2 months of dosing.

CONCLUSIONS:

Rhesus monkeys orally dosed with 0.40 and 2.00 mg/kg/day of Dowco 179 (chlorpyrifos) showed depressions in plasma cholinesterase activity to 41 and 19 percent of control, respectively, after one week of dosing. These activities remained depressed for the remainder of the 6 month study. Erythrocyte cholinesterase activity was also depressed in these two groups (67 - 85 and 13 - 62 percent of control, respectively); brain cholinesterase activity was not affected. Cholinesterase activity was not affected at the lowest dose tested (0.08 mg/kg). No effects were observed on the animals' behavior and appearance, body weight gain, hematology, and clinical chemistry, nor were any gross or histopathologic effects attributable to the test material. However, a limited number of animals were subjected to pathologic examinations. The results were adequate to identify a NOEL and LEL of 0.08 and 0.40 mg/kg, respectively, for effects on cholinesterase activity in the blood. However, the results were not adequate to assess any potential pathologic effect of the test material.

CORE CLASSIFICATION: Supplementary.

This core classification is based on the limited number of animals examined for possible pathologic effects to allow an adequate assessment of the test material's subchronic toxicity.

EPA: 68-01-6561 003822
TASK: 43
April 4, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS

Metabolism

CITATION: Coulston F, Golberg GL, Abraham R, Benitz KF, Griffin TB, Norvell M. 1971. Final report on safety evaluation and metabolic studies on Dowco 179 (IN 151). (Unpublished study received September 18, 1980, under 464-557; prepared by Albany Medical College, Institute of Experimental Pathology and Toxicology, submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:099643-B).

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DATA EVALUATION RECORD

STUDY TYPE: Metabolism in rats and monkeys.

CITATION: Coulston F, Golberg GL, Abraham R, Benitz KF, Griffin TB, Norvell M. 1971. Final report on safety evaluation and metabolic studies on Dowco 179 (IN 151). (Unpublished study received September 18, 1980, under 464-557; prepared by Albany Medical College, Institute of Experimental Pathology and Toxicology, submitted by Dow Chemical U.S.A., Midland, Mich,; CDL:099643-B).

ACCESSION NUMBER: 240090306.

MRID NUMBER: 0043244.

LABORATORY: Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY 12208.

TEST MATERIAL: The test material was identified as "Technical Dowco 179", a tradename for chlorpyrifos. Dowco 179 labeled with carbon 14 on the pyridyl moiety (specific activity, 10.6 mCi/mmol, purity not specified) was provided by Dow Chemical Co.

PROTOCOL:

1. Preliminary studies were conducted with the carbon 14-labeled Dowco 179 and carbon 14-labeled 2,3,6-trichloro-2,6-pyridinol (TCP, presumed to be a major metabolite). Labeled Dowco 179 (10 µCi/rat) was injected intraperitoneally into rats which had been pretreated with 2.0 mg/kg/day of Dowco 179 for 10 days. Samples of several tissues were collected and analyzed. These studies determined that the extraction and analysis techniques did not produce consistent results; the method of extraction was subsequently somewhat improved and used in a second experiment utilizing a single dose of labeled Dowco 179 (2 mg/kg) given intraperitoneally. The carrier vehicle for injection was not specified.

2. To determine the extent of urinary excretion of Dowco 179 in rats, urine was collected over a 44-hour period from 3 male and 3 female Sprague-Dawley rats which had been consuming 0.75 mg/kg/day of Dowco 179 in the diet for 2 months. These animals were part of a 6-month feeding study that is reviewed in a separate DER. A second set of samples was obtained over a 24-hour period a few days later.

After 4 months of dosing, urine was again collected over an unspecified period from 4 females of this dose group. The samples were extracted with benzene and the extracts analyzed for Dowco 179 and derivatized TCP by gas chromatography using a sodium thermionic detector.

3. To study urinary excretion of Dowco 179 in monkeys, urine was collected over 24 hours from 7 Rhesus monkeys that had been dosed via gavage with Dowco 179 for 16 weeks. These animals were part of a 6-month oral toxicity study that is reviewed in a separate DER. Urine samples were collected from the following animals: 1 male and 2 females at the 2.00 m/kg dose level, 1 male and 1 female at 0.40 mg/kg, and 1 male and 1 female at 0.08 mg/kg. The samples were extracted and analyzed as in the rat study.

RESULTS:

The data from the second Dowco 179 tissue distribution study were quite variable for the liver, and therefore the other organs were not analyzed. Averaged values for hepatic Dowco 179 (µg/g fresh weight) were 7.4, 2.6, 0.9, 1.3, and 0.93 for 1, 2, 4, 8, and 24 hours after injection as measured by gas chromatography. Values were not corrected for low extraction efficiency (55 percent in spiked liver samples).

After 2 months of dietary dosing, the rats were found to excrete an average Dowco 179 concentration of 0.012 µg/ml (range 0.005–0.020 µg/ml) which was equivalent to an average total excretion of 0.21 µg in 44 hours and 1.6 µg/ml TCP (range 0.38–5.20 µg/mg), equivalent to an average total excretion of 76.7 µg Dowco 179 per rat. A repeat collection a few days later on the same animals over a 24-hour period found no Dowco 179 and an average of 0.58 µg/ml TCP (range 0.1–1.1 µg/ml), which was equivalent to a total excretion of 12.6 µg Dowco 179 over 24 hours. At 4 months, analysis found that the urine contained no Dowco 179 and an average of 3.0 µg/ml TCP (range 2.4–3.9 µg/ml).

In the study with monkeys, no Dowco 179 was identified in the urine samples collected at 16 weeks. The average TCP concentrations were 0.38 µg/ml (low-dose group), 4.1 µg/ml (mid-dose group), and 18.6 µg/ml (high-dose group). These concentrations were equivalent to an average total excretion for 24 hours of 0.13, 0.58, and 3.00 mg Dowco 179 per animal, respectively.

DISCUSSION:

This study had many deficiencies in design and methodology. The data from the tissue distribution study are rendered almost useless due to high variability and the low extraction efficiency of the method used. At best, the results suggest a rapid elimination phase in the liver through the first 4 hours, followed by a negligible decline through 24 hours. Too few animals were sampled at too few intervals to provide meaningful data

on the excretion of Dowco 179 and its metabolite, TCP. The data presented on urine volume indicated wide variation in the urinary rate in both rats and monkeys, and thus wide variation in the concentration and total excretion rates of the compounds of interest was found. Furthermore, comparison of the 3 analyses in rats indicated wide intertrial variation, suggesting possible difficulties with the analysis method. No limit of detection nor extent of recovery was reported for the method. The excretion of TCP from rats in the 24-hour urine collection sample is only about 2 percent of the 24-hour intake of Dowco 179. Since it can be presumed that TCP is the major metabolite of Dowco 179, further questions concerning the adequacy of the analytical, method can be raised, particularly the efficiency of the TCP derivatization step. Also the levels of other metabolites were not determined. Overall, this study provided very little useful information.

CONCLUSIONS:

Due to deficiencies in design and methodology, this study provided very little useful information on the metabolism of Dowco 179 in rats and monkeys.

CORE CLASSIFICATION: Invalid.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

003822

EPA: 68-01-6561
TASK: 43
March 6, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS

Mutagenicity

CITATION: Kawachi T., et al. 1978. Technical development of screening carcinogens having mutagenicity (an annual report of the National Cancer Center of Tokyo, Japan).

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003822

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity.

CITATION: Kawachi T., et al. 1978. Technical development of screening carcinogens having mutagenicity (an annual report of the National Cancer Center of Tokyo, Japan).

ACCESSION NUMBER: 251678.

MRID NUMBER: Not available.

LABORATORY: National Cancer Center Laboratories of Tokyo Japan, affiliated National Laboratories, and Universities at Kobe, Hokkaido, Hiroshima, Osaka, Tokyo and Sliga (Japan).

TEST MATERIAL: Chlorpyrifos. Details on the purity of the test material were not given.

PROTOCOL:

The report included the data on 25 chemicals which were selected for mutagenicity testing by the National Cancer Center, Tokyo, Japan in 1978. These were summary reports by 14 different investigators, of which four actually reported their results on chlorpyrifos. In all reports, the data were qualitative and the descriptions of the protocols were abbreviated. The following is a listing of the study titles and authors with an abbreviated description of the methodologies used.

1. Kada, T. "Screening of carcinogenic substances by microorganisms" (National Hygienic Laboratory).

   The DNA-damaging test using B. subtilis strains H17 (rec^+) and M45 (rec 45) in the spot assay was employed. The test chemical, 78-15 [a designation for chlorpyrifos], was introduced into a circular filter paper disc (8 mm in diameter); and the two test strains were streaked on the disc. The zones of growth inhibition for the wild type and the rec^- strain were compared for selective inhibition.

2. Sugiyama, T. "Detection of mutagenicity and carcinogenicity by the chromosome aberrations in bone marrow cells of the rat." (Kobe University Medical School).

The test material was injected into the abdominal cavity of Long-Evans male and female rats in physiological saline, containing 0.3 percent pluronic F68 at doses of 1/2, 1/4 and 1/8 of the LD_{50} using at least two animals per group. The bone marrow was taken and slides prepared. Chromosome aberrations were evaluated in 175 fission intermediate cells.

3. Ishidate, M. "Chromosome aberration inducing test using cultured cells." (Mutagenicity Department of National Hygienic Laboratory).

   Chinese hamster cells (CHL) were treated with and without rat liver S-9 activation, and the chromosome aberrations were classified and enumerated. The types of structural aberrations included gaps, breakage, "transformation" [translocation], ring formation, and fragmentation.

4. Yataro, T. "Screening of carcinogenesis using silkworms" (National Genetics Laboratory).

   Silkworm gametocytes were treated with the test material and specific loci controlling egg color (plasma membrane) were screened. The loci used were pe (white egg) and re (red eggs). The wild types (108 and F) were administered the test material by injection. In addition, the authors stated that "Sperms were also used for testing agricultural products (78-11, -14, -15, and -19)."

RESULTS:

1. The test material, chlorpyrifos, at a maximum concentration of 20 mg per disc did not give a DNA-damaging effect in B. subtilis either with or without S-9 activation.

2. Chlorpyrifos was negative in the acute [toxicity] test and at 25 and 50 mg/kg with a [chromosome aberration frequency] value of 1.14 percent. The negative control and positive control results were not included in this report.

3. Chlorpyrifos was negative for induction of chromosomal aberrations in Chinese hamster cells in the absence of rat liver S-9 activation, but was positive in the presence of S-9 after treatment with 0.125 mg/ml chlorpyrifos for 24 hours. The chromosome aberrations were reported to occur at a frequency of about 1 percent. No D_{20} (dose in mg/ml at which 20 percent aberrations are expected) was calculated.

4. In the silkworm assay, there was no mutagenic response reported for chlorpyrifos. However, the general toxicity of this compound at doses below 100 µg was reported to be quite high.

DISCUSSION:

In the four studies where chlorpyrifos was included, this compound was reported to give a negative mutagenic response in all assays except the Chinese hamster CHL cells in the presence of S-9 activation. In addition, it was reported to have a "powerful" toxicity in the silkworm assay.

Unfortunately the reports from all of the studies are summaries of the results and hence do not contain details of the methods used, the purity of the test compound or the study results. Because of the limited information available, an adequate scientific evaluation of this data is not possible.

CONCLUSIONS:

Although the limited information presented in these studies does not permit clear conclusions, at least one result (treatment of CHL cells with the test material in the presence of S-9) suggests that chlorpyrifos may possess mutagenic activity.

CLASSIFICATION: Unacceptable in the present form.

If primary data is submitted for evaluation, the core classification of the studies will be reconsidered.

003822

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK 8232
April 4, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS

Subchronic Oral Toxicity - Rats

CITATION: Coulston F, Golberg L, Abraham R, Benitz KF, Griffin TB, and Norvell M. 1971. Final report on safety evaluation and metabolic studies on Dowco 179 (IN 151). (Unpublished study received December 18, 1980 under 464-557; prepared by Albany Medical College, Institute of Experimental Pathology and Toxicology, submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:099643-B).

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70

been sampled. Also, too few animals (5 per sex in the control and high dose groups) were examined for possible pathologic changes. Overall, the limited examination of the animals precluded detection of potential dose-related effects.

CONCLUSIONS:

Administration of 0.75 mg/kg/day of Dowco 179 (chlorpyrifos) to Sprague-Dawley rats for 6 months resulted in depressions of 50 percent in erythrocyte cholinesterase activity in both male and female animals and in plasma activity in females. No effects were observed on brain cholinesterase activity, nor were effects noted on mortality, weight gain, hematology, clinical chemistry, or gross and histopathology. However, the limited number of animals examined precluded detection of possible effects on cholinesterase activity or other parameters at lower doses. The results of this study suggested a NOEL and LEL of 0.15 and 0.75 mg/kg, respectively, based on plasma and erythrocyte cholinesterase effects.

CORE CLASSIFICATION: Supplementary.

This core classification is based on the fact that the limited number of animals examined in the study precluded detection of possible effects on cholinesterase activity or other parameters at lower doses.

Clinical Chemistry: No dose-related trends were evident in any of the parameters with one possible exception. SGPT levels in the females rats of the mid- and high- dose groups at 6 months were elevated 80 percent over controls; no such trend was noted for the males. Clinical chemistry data for 2 and 4 months were not presented; data for month 2 was reported as lost.

Cholinesterase Activity: No effects on brain cholinesterase activity were evident. Erythrocyte cholinesterase activity was depressed by approximately 50 percent in both male and female animals of the high-dose group at the termination of the study (Table 1). A similar depression was noted in the plasma cholinesterase activity of the high-dose female animals. These data were not statistically compared.

Table 1. Plasma and Erythrocyte Cholinesterase Activity[a] in Blood of Rats Receiving Dowco 179 for 6 Months

Specimen	Sex	Control	0.75 mg/kg
Plasma	Males	0.4 ± 0.1	0.3 ± 0.1
	Females	1.1 ± 0.3	0.5 ± 0.1
Erythrocyte	Males	1.9 ± 0.2	0.9 ± 0.0
	Females	1.9 ± 0.0	1.0 ± 0.2

[a]Activity is expressed as μmoles AcnE hydrolyzed/min; mean and standard deviation of 3 animals.

Gross and Histopathology: There were no significant differences between the control and high-dose animals in the type or severity of any of the gross or histopathologic changes that were observed. All changes appeared to be spontaneous and not a result of test material consumption.

DISCUSSION:

The conclusions drawn from this study are seriously compromised by the limited numbers of animals examined for possible effects. Although the study groups consisted of 20 animals per sex, only 5 animals per sex were examined for effects on hematologic or clinical chemistry parameters. Furthermore, although the test material was known as a cholinesterase inhibitor, only 3 animals were sampled from each group for determination of cholinesterase activity. These small sample sized decreased the sensitivity of the study to identify treatment related effects. For example, although depression of erythrocyte cholinesterase activity was noted in the high-dose females, inhibition of plasma cholinesterase may have been observed in the males and at lower levels if more animals had

3. Observations for signs of toxicity, changes in behavior, and the appearence of tumors were made and recorded daily. Individual body weights and food consumption were recorded weekly.

4. At 2, 4, and 6 months, blood samples were obtained from 5 rats/sex/group and erythrocyte and leukocyte counts, hemoglobin, and hematocrit determined. Differential leukocyte counts were performed in samples having total leukocyte counts of less than $5x10^3$ or more than $10X10^3$. A second blood sample from the same animals was analyzed for the following clinical chemistry parameters: glucose, sodium, potassium, and SGPT.

5. Plasma and erythrocyte cholinesterase activities were measured in samples of blood from 3 animals/sex/group from the control and high-dose groups at 3, 5, 7, and 16 weeks, and from all groups at 6 months. Brain cholinesterase activity was determined on the animals sacrificed at 3 and 6 months (see below).

6. Five rats from each sex and group were sacrified at 3 months; the remainder were sacrificed at the end of the study (6 months). The animals were examined for gross pathology, and tissue samples were preserved and examined for histopathology: heart, trachea, lungs, esophagus, stomach, large and small intestine, liver, pancreas, kidney, urinary bladder, gonads, prostate, uterus, pituitary, thyroid, adrenal, spleen, thymus, lymph node, central nervous system, and eye.

7. Urine was collected from 4 male and 3 female animals of the high-dose group at 2 months and 4 high-dose group females at 6 months over 24-44 hour periods. These samples were analyzed for Dowco 179 and 3, 5, 6-trichloro-2-pyridinol (a major metabolite) by gas chromatography. The results are reported in a separate DER.

RESULTS:

Clinical Observations and Mortality: No signs of toxicity were reported for any of the animals. A single high-dose group male was observed to have paralysis of the hind legs in the 5th month and was sacrificed. A total of 16 rats were found dead during the study; all deaths were attributed to chronic murine pneumonia which was diagnosed at necropsy. The distribution of mortalities was not related to dose.

Body Weight and Food Consumption: Body weight gain and food consumption were similar among the males and females of all groups throughout the course of the study.

Hematology: No dose-related trends were evident in any of the parameters measured at 2, 4, or 6 months. It should be noted that, with the exception of one value, the hematology data for month 4 are identical to the month 6 data when these latter data are rounded off.

003822

DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral toxicity - rats.

CITATION: Coulston F, Golberg L, Abraham R, Benitz KF, Griffin TB, and Norvell M (IN 151). 1971. Final report on safety evaluation and metabolic studies on Dowco 179. (Unpublished study received September 18, 1980 under 464-557; prepared by Albany Medical College, Institute of Experimental Pathology and Toxicology, submitted by Dow Chemical U.S.A., Midland, Mich.; CDL:099643-B).

ACCESSION NUMBER: 240090306.

MRID NUMBER: 00043244.

LABORATORY: Institute of Experimental Toxicology and Pathology, Albany Medical College, Albany, NY 12208.

TEST MATERIAL: The test material was identified as "Technical Dowco 179," a tradename for chlorpyrifos. The source was not specifed. Analysis of diets: Analysis data were reported for the 3 dose levels for approximately 23 dates during the study. The results of the analysis (reported as ppm), body weight, and food consumption data were used by the reviewer to verify that the desired dose levels were achieved. At the points that were checked, the actual dosage was within 15 percent of the desired dosage. Stability in the diets: No information was provided on the stability of the test material in the diets.

PROTOCOL:

1. One hundred and sixty Sprague-Dawley rats, 80 of each sex, weighing 90-100 g, were divided into four groups (20 animals/sex/group) for this study. The rats were individually housed in environmentally-controlled quarters and were provided a commercial laboratory ration and water ad libitum. The source of the animals was not specified.

2. The test material was administered in the diet for 6 months in proportions estimated to produce dose levels of 0.0, 0.03, 0.15, and 0.75 mg/kg/day. Diets were prepared weekly by adding the test material in an acetone solution to the feed in a Hobart mixer, and the test material content was adjusted according to food consumption to achieve the desired dosages.

003822

DATA EVALUATION RECORD

CHLORPYRIFOS

Unscheduled DNA Synthesis

MRID: 00043657.

CITATION: Mitchell AD. 1975. Unscheduled DNA synthetic testing of substitute pesticides. Pages 151-153, In Substitute chemical program. The first year of progress: Vol. II. Toxicological methods and genetic effects workshop: proceedings of a symposium, Wasnington, D.C. U.S. EPA, OPP, ORD.

REVIEWED BY:

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CORE CLASSIFICATION: Unacceptable.

This study could not be fully evaluated because it contains only summary information.

003822

DATA EVALUATION RECORD

CHLORPYRIFOS

Gene Mutations in Bacteria

MRID NUMBER: 05004861.

CITATION: Poole DC, Simmon VF, Newell GW. 1977. In vitro mutagenic activity of fourteen pesticides. Toxicol. Appl. Pharmacol. 41:196.

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CORE CLASSIFICATION: Unacceptable.

This study could not be fully evaluated because it is an abstract.

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003822

DATA EVALUATION RECORD

CHLORPYRIFOS

Gene Mutations in Bacteria

MRID NUMBER: 05010438.

CITATION: Simmon VF, Poole CD, Newell GW. 1976. In vitro mutagenic studies of twenty pesticides. Toxicol. Appl. Pharmacol. 37:109.

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CORE CLASSIFICATION: Unacceptable.

This study could not be fully evaluated because it is an abstract.

003822

DATA EVALUATION RECORD

Chlorpyrifos

Cholinesterase

CITATION: Dow Chemical U.S.A., 1971. Studies on human exposure to Chlorpyrifos. (Unpublished study received September 15, 1978 under 270-EX-1; submitted by Farnam Cos., Inc., Phoenix, Ariz.; CDL:235685-D).

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Signature: Gary J. Burin
Date: 5/18/84

CORE CLASSIFICATION: Invalid. This report summarized data from previous studies; it contained no original data to evaluate.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

003822

EPA: 68-01-6561
TASK: 43
March 6, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS

Mutagenicity/Embryo Toxicity

CITATION: Bloom SE, Muscarella DE, and Schaefer DP. 1981. Toxicological Evaluation of the Insecticide Chlorpyrifos: Assays for Genetic Damage and Developmental Toxicology; a report prepared by the Department of Poultry and Avian Sciences, Cornell University, Ithaca, NY for Eastern Artificial Insemination Coop., Inc., Ithaca, NY.

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APPROVED BY:

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity/Embryotoxicity.

CITATION: Bloom SE, Muscarella DE, and Schaefer DP. 1981. Toxicological Evaluation of the Insecticide Chlorpyrifos: Assays for Genetic Damage and Developmental Toxicology; a report prepared by the Department of Poultry and Avian Sciences, Cornell University, Ithaca, NY for Eastern Artificial Insemination Coop., Inc., Ithaca, NY.

ACCESSION NUMBER: 251679.

LABORATORY: Department of Poultry and Avian Sciences and Diagnosis Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

TEST MATERIAL: The test materials were identified as chlorpyrifos O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate and its two metabolites 3,5,6-trichloro-2-pyridinol and diethyl-3,5,6-trichloro-2-pyridyl phosphate. These materials were "supplied as pure compounds by Dow Chemical Co."

PROTOCOL:

Controls. The solvent control for the test chemicals was acetone; the positive control compounds were ethyl methanesulfonate (EMS) and methyl methanesulfonate (MMS).

Genetic Assays.

Chick Embryo In Vivo Cytogenetics. Eggs of Cornell K-strain were incubated at 37.5° C for 70 hr, and were candled to select for normal and healthy embryos. A 0.5 cm^2 portion of the shell which was over the air cell was removed so that the test solutions could be applied.[1] At 72 hours, 150 ug of 5'-bromodeoxyuridine (5'-BrdU) was injected onto the inner shell membrane and 2 hours later, the test compound or control substance were injected similarly in 10 ul of acetone. Selection of the dosage levels used for mutagenicity tests was based on acute and chronic toxicity assays run on 3 day old Cornell K-strain embryos. Chlorpyrifos was administered at dosages of 1.11, 11.1, 111.0 and 1110.0 ug [per egg].

[1]Bloom, SE and Hsu TC. 1975. Chromosoma 51:251-267.

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Pyridyl phosphate was administered at dosages of 0.10, 1.00, 10.0 and 50 ug [per egg] and pyridinol at dosages of 1.11, 11.1, 55.5 and 555 ug [per egg]. Twenty-two hours after injection with BrdU, 20 ul of 0.05 percent colecemid was injected. After a two hour incubation with colcemid, the embryos were removed, treated with 0.9 percent sodium citrate for 30 min., and fixed in ethanol:acetic acid (3:1) for 24 hr at 7° C. Chromosome preparations from the allantoic sac or limb buds were made using a solid tissue technique.[2] Hoechst 333258 (0.5 ug/ml) was applied to slides for 15 min., and the slides were rinsed, air dried, mounted and examined by fluorescence microscopy. The SCEs [visualized by harepin chromosome configuration] were scored where possible in 25 metaphase plates from eight embryos at each treatment level with chlorpyrifos. For the metabolite pyridyl phosphate, only 6 embryos per treatment level were examined and for pyridinol, 9 embryos were examined at the dosage of 11.1 ug.

Chinese Hamster Ovary (CHO) In Vitro Cytogenetics. The preliminary toxicity assays formed the basis for dosages of chlorpyrifos, pyridyl phosphate and pyridinol used in this assay. The concentrations employed were 1.0, 10.0 and 100 ug per ml. The solvent control was acetone and 8.6 ug/ml MMS served as the positive control.

CHO cells were grown in tissue culture flasks (25 cm^2) that contained five ml Eagle's Minimum Essential Medium (MEM) fortified with 10 percent fetal calf serum (FCS), penicillin/streptomycin and non-essential amino acids. Each flask was inoculated with 5×10^5 CHO cells and incubated in 5 percent CO_2 95 percent relative humidity at 37° C overnight. At each treatment level, the test material was added to four replicate cultures in 100 ul of the stock solution at an appropriate level. After treatment for four hours the cells were washed with phosphate buffered saline and fresh culture medium was added. Two treated cultures were used for SCE analyses and two for analysis of chromosomal aberrations. Positive (MMS) and negative (acetone) controls were treated similarly.

Cultures used for SCE analysis received 5 ug/ml BrdU in flasks wrapped in aluminum foil to prevent photolysis. All cultures were incubated for 24 hr, after which time 0.1 ug/ml colcemid was added. Incubation was continued for 4 hr and the flasks were shaken to elute metaphase cells. The eluted cells were treated with 0.45 percent sodium citrate in water at 37° C for 20 min, fixed in methanol:glacial acetic acid (3:1) for 30 min, resuspended in fresh fixative and refrigerated overnight. These cell suspensions were used to prepare slides for analyses of chromosome aberrations (staining with 4 percent Giesma) and for SCE (flourescence plus giemsa staining)[3]. For SCE twenty-five metaphases per culture were scored and for chromosome aberrations 100 cells, if available, were evaluated.

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[2]Bloom SE. 1978. In: Chemical Mutagens; Principles and Methods for their Detection, Vol 5, A. Hollaender and F.J. deSerres (eds.) Plenum Press, New York, pp. 203-232.

[3]Byrnes, JA and Bloom, SE. 1980. Mutat. Res. 70:203-210.

Bovine Blastocysts Assay. Thirteen Holstein cows, synchronized in their estrous cycle and superovulating, were divided randomly into groups and inseminated either with semen collected from one untreated control bull or one of four bulls treated with Dursban 44 [unspecified concentration]. The experiment consisted of two trials. In the control, the same bull and cows were used. In the Dursban group each cow was inseminated by semen of two treated bulls so that blastocysts fertilized by a total of four bulls could be examined. All of the four treated bulls "displayed severe clinical symptoms of poisoning."

Blastocysts were collected on day eight following insemination for trial 1 and on day seven for trial 2 "by flushing the uterus of each cow with 1 liter of Dulbecco's Modified Eagle's Medium containing 1 percent FCS, 25 mM Hepes buffer, sodium pyruvate, and 1,000 ml/l glucose." The flushed material was collected, allowed to settle for 30 min in a 1 liter graduated cylinder at room temperature, and the lower 100 ml retrieved and scanned for blastocysts. After elimination of unfertilized ova and degenerating zygotes, the viable blastocysts were transferred to a 35 mm tissue culture dish containing 2 ml of the medium described above which was fortified with 0.05 ug/ml colcemid and 15 percent FCS. The suspension was incubated at 37° C/ for 1 hour, then the blastocysts were transferred to a water /FCS solution (3:1) for 15 min, and fixed in 3:1 methanol:acetic acid for 1 hour. Blastocysts were pipitted onto glass slides, covered with one or two drops of a 1:1 mixture of methanol:acetic acid fixative and 50 percent glacial acetic acid, and air cried at room temperature. Four percent Giemsa was used to stain the slide preparations of blastocysts. Each blastocyst was examined for metaphase cells, ploidy, chromosome morphology, and sex of the zygote.

Statistics. One-way analysis of variance and the t-test were applied to the SCE data from chick embryo and CHO assays.

RESULTS:

Chick Embryo Assay. Chlorpyrifos treatment at 1,110 ug and 2,220 ug resulted in significant embryonic death, 8/22 and 9/13, and significant reduction of embryonic weight at 17 days. A treatment at 111 ug of pyridinol or pyridyl phosphate resulted in the death of 63.6 and 81.8 percent of the embryos, respectively. There were no significant weight reduction in the 17 day old embryos that survived this treatment. None of the test compounds produced a SCE level higher than in controls.

The mean number of SCEs in the solvent (acetone control was 1.24 ± 1.02 for 150 cells. At 1,110 ug of chlorpyrifos the mean number of SCEs per cell for 165 cells was 1.47 ± 0.99 [virtually the same results was seen for all other treatment levels]. Treatment with 240 ug EMS (positive control) induced a mean of 5.11 ± 2.49 SCEs per cell in 131 cells.

In the chick embryo assay at 60 ug pyridyl phosphate, there was a mean of 1.65 ± 1.15 SCEs per cell from 63 cells analyzed, compared to 1.59 ± 1:22 SCEs per cell from 150 cells analyzed in the solvent control. At doses of 0.10, 1.00 and 10.0 ug of pyridyl phosphate, the SCE values were also similar to the control.

At doses of 55.5 and 555 ug of pyridinol the mean SCE/cell values in chick embryo cell were 2.02 ± 1.34 for 127 cells analyzed and 2.24 ± 1.33 for 25 cells, respectively. At pyridinol concentrations of 1.11 and 11.1, the mean values for SCEs/cell were similar. The solvent control for this assay gave a mean SCE/cell value of 1.77 ± 1.29 from 141 cells and the value for EMS treatment was 7.94 ± 1.78 in 16 cells analyzed.

CHO Assays. Assays for SCEs and chromosome aberrations were conducted for chlorpyrifos and its metabolites, pyridyl phosphate and pyridinol. The results for SCE induction are summarized as follows: For chlorpyrifos treatments at the various dose levels, SCEs per cell were 1.0 ug/ml (6.52 ± 2.57), 10 ug/ml (7.02 ± 3.24) and 100 ug/ml (6.8 ± 2.81), acetone control (7..00 ± 2.51) and MMS (30.52 ± 7.39). For 3,5,6-Trichloro-2-pyridinol at the various dose levels, SCEs per cell were 1.0 ug/ml (6.86 ± 2.98), 10.0 ug/ml (6.44 ± 2.78), 100 ug/ml (6.66 ± 2.67), acetone control (7.00 ± 2.62) and MMS (25.32 ± 7.34). For diethyl-3,5,6-trichloro-2-pyridyl phosphate at the various dose levels, SCEs per cell were 1.0 ug/ml (6.20 ± 2.89), 10.0 ug/ml (6.44 ± 2.57), 100.0 ug/ml (6.46 ± 1.97), acetone control 6.40 ± 2.28) and MMS (22.20 ± 5.57). For each assay compound, 60 cells were scored at each dose and the data was pooled from two replicate cultures. The values for MMS treated cells in each assay were significantly different from the control at $p < 0.001$; however, none of the treatments with chlorpyrifos or the metabolites gave SCE/cell values that were statistically different from their respective controls.

The chromosome aberrations induced by chlorpyrifos, pyridyl phosphate and pyridinol are summarized in Table 1. There was no apparent differences in the incidence of abberation between any treated or control group. The majority of abberations were chromatid gaps and breaks and chromosome gaps.

Bovine Blastocysts. The bovine blastocysts assay was conducted with the commercial product, Dursban 44 which is described[4] as "emulsifiable concentrate (2 and 4 pounds/gallon), granular and dusts." The method of application was not described, but the authors stated that all of the four Dursban treated bulls "displayed severe clinical symptoms of poisoning following exposure to Dursban 44."

From the eight cows inseminated by the control bull sperm in two trials, 51.3 percent of the zygotes were normal blastocysts, 25.6 percent of the zygotes were abnormal, and 23.1 percent of the ova were not fertilized. After treatment with Dursban 44 sperm from the four bulls were used to

[4]Farm Chemicals Handbook. 1981. Meister Publishing Co., Willoughby, OH, pg C7.

TABLE I.* CHO Assay for Chromosome aberration Induction by Chlorpyrifos,[a] Pyridinol,[b] and Pyridinol Phosphate[c]

Dose (ug/ml)	Chromatid gaps			Chromatid breaks			Chromosome gaps			Chromosome breaks			Other			Number of normal cells		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Control (acetone)	10	5	5	1	2	4	4	0	3	0	0	0	0	0	0	185	193	188
1.0	3	4	2	1	1	0	3	2	4	0	0	1	0	0	0	193	193	193
10.0	4	5	4	0	1	1	4	0	2	0	0	0	0	0	0	192	192	193
100.0		4	10		1	2		3	3	--	--	10	--	--	10	0	192	185
MMS	6	11	4	1	2	4	1	5	3	0	0	0	0	0	1	192	182	188

*Adapted from Bloom SE, Muscarella DE, and Schaefer OP. 1981. Toxicological Evaluation of the Insecticide Chlorpyrifos. Assays for Genetic Damage and Developmental Toxicity. The data are average of 200 cells scored in two replicate cultures for each compound at each dose level.

inseminate cows as follows: Bull No. 1 (2), Bull No. 2 (2), Bull No. 3 (4) and Bull No. 4 (4). Of the recovered zygotes 52.6 percent were normal blastocysts, 12.3 percent of the zygotes were abnormal, and 35.1 percent of the ova were not fertilized.

Cytogenetic evaluation of the blastocysts was reported by the authors to reveal no evidence that Dursban 44 caused mutagenic damage in embryos from the inseminated cows. The zygotes from cows inseminated by the control bull produced 14 blastocysts which had a sex ratio of 1 male to 8 females. All except one (a possible tetraploid) were diploid zygotes. The 25 zygotes from the four cows inseminated by the four Dursban-treated bulls blastocysts had a sex ratio of 10 males to 6 females and all except one (a possible tetraploid) were diploid.

DISCUSSION:

The sensitivity of the chick embryo assay and the bovine blastocyst assay to detect a potential mutagen has not been established. These are not widely used tests for sister chromatid exchange or cytologic analysis, and in our opinion, there has been insufficient testing using these assays to establish their usefulness for establishing the mutagenicity of a test substance.

In the present study, a positive control was not included with the bovine blastocysts assay. Therefore, the absence of an effect with the test compounds cannot be adequately judged as a negative response. Although the positive control in the chick embryo assay showed a statistical increase in SCEs compared to controls, this is not as large an effect as would be expected in a rodent system using a strong mutagen/clastogen like EMS. In the CHO test for SCE, the positive control gave the expected result but chlorpyrifos and its metabolites caused no increases in SCEs compared to control. When chromosomal aberrations were scored in the CHO assay, the positive control (MMS) caused fewer aberrations than solvent in 2 of the 3 assays and only a small increase in the third assay. A negative effect with chlorpyrifos and its metabolites, therefore, is not interpretable.

The only information from this study which might be useful in assessing the potential hazardous nature of chlorpyrifos or its metabolites was the embryotoxic and acute toxicity of chlorpyrifos and its metabolites in the chick embryo assay. Proof is lacking that any of the assays could determine if a mutagen was detoxified after *in vivo* exposure, or if an indirect as well as direct acting mutagen could be detected. It was claimed by the authors that the tests selected were capable of both.

However, none of the assays appeared to have the required sensitivity to detect a positive mutagenic or clastogenic response.

CONCLUSIONS:

Under the conditions of these assays the results do not permit an assessment of the potential mutagenic or clastogenic properties of chlorpyrifos, pyridinyl phosphate and pyridinol.

CLASSIFICATION: Unacceptable.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12356)

003822

EPA: 68-01-6561
TASK: 43
April 4, 1984

DATA EVALUATION RECORD

CHLORPYRIFOS

Subchronic Oral in Rats

CITATION: Coulston F, Griffin TB. 1975. A safety evaluation of Dowco 214 in rats and Rhesus monkeys. An unpublished report prepared by Albany Medical College, Institute of Comparative and Human Toxicology and submitted by Dow Chemical, U.S.A., Midland, MI. July 1975.

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APPROVED BY:

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EPA Scientist

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003822

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (six weeks) oral toxicity in rats.

CITATION: Coulston F, Griffin TB. 1975. A safety evaluation of Dowco 214 in rats and Rhesus monkeys. An unpublished report prepared by Albany Medical College, Institute of Comparative and Human Toxicology and submitted by Dow Chemical, U.S.A., Midland, MI. July 1975.

ACCESSION NUMBER: 219701910.

MRID NUMBER: 00028464.

LABORATORY: Institute of Comparative and Human Toxicology. Albany Medical College, Albany, New York.

TEST MATERIAL: Dowco 179 (Chlorpyrifos).

PROTOCOL:

1. This review is an evaluation of a portion of a six-week oral toxicity study that tested three dose levels of Dowco 214 and one dose-level of Dowco 179 in rats. Data for the control and Dowco 179 groups will be addressed; the Dowco 179 group was included in the study as a point of comparison for the toxicity of Dowco 214.

2. Twenty Sprague Dawley rats were randomly assigned to either the dose group or the control group; each group contained 5 males and 5 females. The initial mean (± standard deviation) body weights were 210 (± 14) grams for males and 178 (± 8) grams for females.

3. The test material was suspended in 1 percent gum tragacanth; suspensions were prepared weekly and stored under refrigeration. The Dowco 179 concentration of each preparation was determined by gas chromatography. Single daily doses of 1.0 mg/kg body weight were given by stomach tube six days per week for 6 weeks. Animals were weighed each week and the dosage adjusted accordingly.

4. The animals were caged individually in temperature-controlled quarters and allowed free access to food and water throughout the study.

5. Heparinized blood samples from the orbital sinus were obtained from each rat before the six-week dosing period began and after 30 days of dosing. Cholinesterase activity was determined in the plasma and in erythrocyte hemolysates.

6. After six weeks of dosing, animals were sacrificed by inhalation of ether. Blood samples from the aorta were obtained from each rat for the evaluation of clinical chemistry and hematologic parameters. Determinations included hemoglobin concentration, packed cell volume, erythrocyte and leucocyte counts, total serum protein, bilirubin, glucose, urea nitrogen, glutamic-oxaloacetic transaminase activity, and lactic dehydrogenase activity.

7. The animals were not examined for gross abnormalities and no tissue samples were collected or examined for histopathologic changes.

8. Liver samples were obtained from each animal for the determination of the N-demethylase and biphenyl hydroxylase activities.

9. Homogenates of whole brain from each animal were assayed for cholinesterase activity.

10. The data were not statistically analyzed.

RESULTS:

Dowco 179 at a dose of 1.0 mg/kg had no effect on body weight, clinical blood chemistry and hematology values, and on the activity of mixed function oxidases of the liver.

Plasma and erythrocyte cholinesterase mean (± standard deviation) activity data are shown in Table 1. The report stated that after dosing, the plasma cholinestease activity in males and females and the erythrocyte cholinesterase activity in females showed significant decrease; however, there was no indication that the data were statistically analyzed.

Brain cholinesterase activity was reported as not significantly different from the control for males and females.

TABLE 1. Mean (± Standard Deviation) Activity of Plasma and Erythrocyte Cholinesterase in Rats Before and after 30 Days of Oral Dosing

Group	Male		Female	
	Before	After	Before	After
Plasma Cholinesterase[a]				
Control	0.9(0.1)	0.5(0.1)	1.8(0.9)	2.4(1.5)
Dowco 179 (1.0 mg/kg)	0.6(0.1)	0.3(0.04)[c]	1.6(0.2)	1.1(0.2)[c]
Erythrocyte Cholinesterase[b]				
Control	1.1(0.1)	1.9(0.2)	1.6(0.2)	2.1(0.3)
Dowco 179 (1.0 mg/kg)	1.3(0.2)	1.0(0.2)[c]	2.2(0.2)	1.0(0.2)[c]

[a] µmol acetate/min/ml plasma.

[b] µmol acetate/min/ml packed erythrocytes.

[c] Differences in dosed animals from before dosing level and from control level were statistically significant ($p<0.05$); t-statistics were calculated by this reviewer.

DISCUSSION:

Evaluation of this study on the basis of subchronic (6-months) testing criteria indicated several deficiencies; these involved the duration of dosing, the number of dose levels tested, the number of animals per sex per group, and the failure to examine the animals for gross and histopathologic abnormalities. In addition, it could not be determined that the quantitative data, especially the cholinesterase results, were statistically evaluated in support of final report statements of significant decreases. Therefore, this reviewer conducted t-statistic comparisons of mean differences in the cholinesterase values (Steel and Torie, 1960. Principles and Procedures of Statistics, McGraw-Hill, pp 72 - 80). The results of the statistical analysis (Table 1) indicated that plasma and erythrocyte cholinesterase activities were significantly reduced in both male and female rats when compared either to their respective predosing mean or to the control group mean.

The final report was deficient in that it did not specify whether the animals were fasted before each dose or whether the control group rats received daily gavage doses of the vehicle.

Despite the deficiencies in the protocol and the final report, this study provided useful information on cholinesterase activity, hematologic and clinical blood chemistry parameters, and on body weight changes in rats exposed to chlorpyrifos (Dowco 179) at 1.0 mg/kg for six weeks.

CONCLUSIONS: Daily doses of 1.0 mg/kg of chlorpyrifos by gastric intubation for six weeks produced statistically significant ($p<0.05$) decreases in plasma and erythrocyte cholinesterase activity in male and female Sprague-Dawley rats. Only one dose-level was tested, therefore, the NOEL and LEL could not be derived.

CORE CLASSIFICATION: Supplementary data.

The dosing period was limited to 6 weeks, only one dose level was tested, and no patnology data were collected.

END