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1-27-87

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

005685

JAN 27 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Expedited Review of Chlorpyrifos 90-Day Rat Inhalation Study.
EPA ID #464-404, Accession No. 400139, Caswell #219AA, Tox. PN 7-0237

TO: Dennis Edwards (12)
Reg. Div. (TS-767C)

FROM: Winnie Teeters, Ph.D. W. Teeters, 1-22-87
Pharmacologist. Section V
Tox./HED (TS-769C)

THRU: Laurence D. Chitlik, D.A.B.T. Laurence D. Chitlik 1-22-87
Head, Section V
Tox./HED (TS-769C)
and
Theodore M. Farber, Ph.D. for CDB 1-22/86
Chief, Toxicology Branch
Hazard Evaluation Div. (TS-769C)

Action Requested:

Review the 90-day, nose-only inhalation study in rats with chlorpyrifos within an expedited time-frame.

Recommendations:

1. This subacute, nose-only inhalation study (ID # K-044793-077) is a completely negative study with no compound related effects noted in Fisher 344 rats exposed to 0, 5.2, 10.3 or 20.6 ppb chlorpyrifos 6 hrs./day, 5 days/week for 13 weeks. (The sponsor stated that the theoretical maximum vapor concentration of chlorpyrifos is 25 ppb at 25 degrees centigrade.) Consequently, the NOEL in this study is 20.6 ppb (287 ug/m^3), HDT, and the LEL was not established.

2. The unusual aspect of this study is the lack of an effect on cholinesterase activity of plasma, erythrocytes or brain. This is remarkable since in a whole-body inhalation exposure of 14 day's duration at only 0.7 ppb concentration, there was 15% depression of plasma cholinesterase in female rats. Three possible explanations are discussed. Any information the sponsor may provide to explain the difference would be helpful. Please refer to the "Discussion" on page 4A and 5 of this review for more information.

3. The sponsor did not provide detailed descriptions of the exposure chambers so that location of the sampling port in relationship to exposure ports of the chambers was clearly defined. However, there was a statement to the effect that chamber distribution studies showed that exposure concentrations varied less than 15% between sampling and exposure ports, but supporting data were not provided.

The sponsor is requested to submit a thorough, detailed description of the exposure equipment and the data developed during chamber distribution studies. Classification of the study will be made after receipt and review of this material.

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Reviewed by : Winnie Teeters
Section V, Tox. Brn. (TS-769C)
Secondary Reviewer: Laurence D. Chitlik, D.A.B.T.
Section V, Tox. Brn. (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Inhalation TOX. CHEM. NO. 219 AA

ACCESSION NUMBER: 400139 MRID NO.: - TOX. PROJ. NO. 7-0237

TEST MATERIAL: Chlorpyrifos

SYNONYM: Dursban

STUDY ID: K-044793-077

SPONSOR: Dow Chemical Co.

TESTING FACILITY: Toxicology Research Lab., Dow Chemical Co.

TITLE OF REPORT: Chlorpyrifos: 13-Week Nose-Only Vapor Inhalation Exposure Study In Fisher 344 Rats.

AUTHORS: R.A. Corley et al.

REPORT ISSUED: Nov. 13, 1986

CONCLUSIONS: Nose-only exposure of Fisher 344 rats by the inhalation route to 0, 5.2, 10.3 or 20.6 ppb chlorpyrifos 6 hrs/day, 5 days/week for 13 weeks did not induce changes in body weight or signs of treatment-related toxicity during the study or changes in urinalysis, hematology, clinical chemistry, organ weights, gross pathologic and histopathologic evaluations performed at the end of the study nor alterations in plasma, erythrocyte or brain cholinesterase activities also performed at study termination. Therefore, the NOEL in this study is 20.6 ppb (287 ug/m^3), HDT, in male and female Fisher 344 rats, and the LEL was not established. The sponsor stated that the theoretical maximum vapor concentation of chlorpyrifos is 25 ppb at 25 degrees centigrade.

CLASSIFICATION: The study will be classified after receipt and review of requested material.

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A. MATERIALS:

1. Test compound: Technical chlorpyrifos (phosphorothioic acid 0,0-diethyl - 0-[3,5,6-trichloro-2-pyridinyl] ester). Purity was 100.0 +/- 0.9%. The lot number was AGR 219646.

2. Test animals: Six-week old Fisher 344 rats were received from Charles River and used after an acclimation period of 3 weeks to the laboratory and an additional 4 weeks to the nose-only tubes.

B. STUDY DESIGN:

1. Animal assignment: The rats were assigned to control or exposure groups by the use of a computer-generated random number table. Ten rats/sex/group were exposed to targeted concentrations of 0, 5, 10 or 20 ppb (0, 72, 143 or 287 ug/m_3) chlorpyrifos for 6 hrs/day, 5 days/week for 13 weeks. Animals at the extremes of the body weight distribution were not used.

2. Chambers and vapor generation and analysis: The chambers were 44 liter ADG-design, nose-only exposure types. Airflows were calibrated and maintained at approximately 25 l/min. Chamber temperature and relative humidity were generally recorded at least twice daily. Test atmospheres were generated by passing warmed air through glass pipes containing glass beads coated with chlorpyrifos. Test concentrations were typically determined three times/chamber/day, except for the control chamber, which was analyzed weekly. Analysis was by GLC with a standard curve run daily. It was stated that chamber distribution studies verified that exposure concentrations varied less that 15% (calculated on a worst-case basis by: range x 100/lowest value) between sampling and selected animal exposure ports, but no supporting data were supplied to support this statement.

   The sponsor is requested to provide a thorough, detailed description of the exposure equipment and the data developed during chamber distribution studies.

3. Statistical evaluation: The methods used are described in the following page copied from the report (pages 14 and 15 from the report).

4. Quality Assurance: There was a quality assurance statement which included six dates of inspection.

C. METHODS and RESULTS:

1. Chamber concentrations:

   Methods: Methods of analysis were described above under Study Design.

   Results: The mean and standard deviation for the 65 daily time-weighted averages of chamber concentrations were as follows:

A

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Pages _____ through _____ are not included.

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_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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Targeted conc. (ppb)	Analytical conc. (ppb)
0	None detected by weekly analysis (Detection limit= 0.1 ppb)
5	5.2 +/- 0.8
10	10.3 +/- 1.3
20	20.6 +/- 2.6

2. Observations and body weights:

Methods: All animals were observed following each exposure for evidence of treatment-related effect. Observations on non-exposure days were limited to monitoring for availablilty of food and water. Body weights were recorded prior to the first exposure and approximately weekly thereafter.

Results: One female control rat died apparently from suffocation during the sixth week of the study; no other deaths occurred. During only the first month all rats, including controls, showed a slight red staining around the nose and eyes. Body weights were comparable among all groups (see following Tables 3 and 4, taken from the report); however, all of these groups did not gain as much as rats not subjected to daily restraint for nose-only exposure (mean of means for control males: 360g, range of means for present study: 306-318g for all groups; mean of means for control females: 195g; range of means for present study: 167-169g).

3. Hematology:

Methods: Orbital sinus blood was evaluated for hematocrit, hemoglobin, erythrocyte count, total leucocyte count, platelet count and differential leucocyte count (evaluated only for the high level and control).

Results: Male rats did not show any changes from controls. Female rats of all treated groups showed a slight decrease (<4%: $p<0.05$ for each) in erythrocyte count which was not strictly dose-related and was within the range of historical control data. There were no supporting findings to indicate that this change was compound related. Because of these circumstances this change was conconsidered to be biological variation.

4. Urinalysis:

Methods: Urine collected prior to necropsy was evaluated by "Chemstrip 7" for bilirubin, glucose, ketones, blood, pH, protein and urobilinogen. Specific gravity was determined with a hand-held refractometer.

Results: There were no statistical differences between treated and control rats of either sex for specific gravity values and no notable differences among all of the groups for any of the other urinary parameters. Apparently there is an error in Table 8 of the report for the females since the number for specific gravity values for the control group is 10, yet one rat died.

5. Clinical chemistry:

Methods: Blood samples collected at necropsy were analyzed with a CentrifiChem System for urea nitrogen, alanine aminotransferase activity.

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asparatate aminotransferase activity, bilirubin, alkaline phosphatase activity, glucose, total protein, albumin, globulins, calcium and phosphorus. Sodium, potassium and chloride were measured on a Beckman System E4A Electrolytes Analyzer.

Results: Although urea nitrogen was slightly elevated (about 13%) for all exposed males, the differences from controls were statistically significant ($p<0.05$) only for the low and high dose groups because their variablity was less, even though all treated males had the same mean value (17 vs 15 mg/dl for the control). Females of the low dose group had a minimally increased sodium value which was statistically different ($p<0.05$) from the controls(141, 143, 142 and 142 mmol/l for controls, low, middle and high dose groups, respectively). Neither this sodium value nor the urea nitrogen values are considered to be biologically meaningful differences from the control values and they were not dose-related and were within the range of historical control values.

In Table 10 of the report for the females the number of values for the control group is only 8, yet only 1 female died. Likewise, the number of values for the 5 ppb group of females for bilirubin, calcium and phosphorus is only 9, not 10.

5. Cholinesterase:

Methods: Plasma, erythrocyte and brain cholinesterase activities were determined by a photometric technique (Boehringer Manneheim Diagnostics Set).

Results: All cholinesterase activity values were comparable among treated and and control groups for respective sexes (see following Tables 11 and 12 copied from the report).

6. Pathology:

Methods: After at least 4 consecutive final exposures, surviving rats were sacrificed the day after the last exposure. The rats were weighed, anesthesized with methoxyflurane and had their tracheas clamped prior to decapitation. Brain, lungs, liver, kidneys, adrenals and testes were weighed and all animals were examined for gross pathological changes and a complete set of tissues (see following Table 1 copied from the report) was collected from each animal; these were processed and stained by conventional techniques. Histopathological examinations were performed on rats in the control and high exposure groups.

Results: There were no statistically significant differences from controls for terminal body weights or for absolute or relative organ weights of the treated groups of either sex (see following report Tables 13 and 14). Furthermore, there were no notable changes observed in gross or histopathological examinations of the tissues between control and treated rats.

7. Discussion: This is a completely negative study. The only differences between control and exposed rats were the slighty decreased (<4%) erythrocyte counts for all levels of exposed females, the slightly elevated (approximately 13%) serum urea nitrogen for all levels of exposed males and the minimum increase (<2%) in serum sodium in low level females.

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There was no dose-relationship for any of these findings, none was of notable magnitude and each change was within the range for historical control data. Consequently, these effects are not considered exposure-related nor are they of biological consequence.

This lack of any compound effect is not surprising in view of the low concentrations to which the animals were exposed in relationship to the usual levels of chlorpyrifos which produce systemic toxicity (e.g. >120 ppm in dog feeding studies) except for its cholinesterase-depressant activity. The unusual aspect of this study is that there was no effect whatsoever seen on cholinesterase activity - neither on plasma, erythrocyte, or brain - at the highest level of 20 ppb in this 90-day nose-only study whereas in a whole-body exposure study, a concentration of only 0.7 ppb for 14 days induced a 15% depression of plasma cholinesterase in female rats (memo of Saunders to Ellenberger, Jan. 27, 1986)

There are at least three possible explanations for this apparent discrepancy. One is, as the sponsor suggested, that oral and dermal exposure accompany whole-body inhalation exposure and contribute substantially to the total exposure under this situation. A second explanation can be that in this longer term inhalation study (90 days vs 14 days), accomodation to the cholinesterase depressant effect has occurred (unfortunately, cholinesterase activity of plasma and erythrocytes was not measured in the present study until termination at 90 days).

A third possibility is that the animals did not receive the reported concentrations. No details of the exposure equipment accompanied this report although references were made to a couple published articles on the equipment and its use. Methodology in the report mentioned that "automated sampling of chamber air via solenoid valves was not possible due to adsorption of chlorpyrifos to surfaces and to very low chamber concentrations". One of the specific requests made when the proposed protocol for this study was reviewed was that a "sufficient number of samples must be taken from the breathing zone to demonstrate that all animals within a test group received the same dose of test material" (memo of Saunders to Ellenberger, Jan. 15, 1986). The sponsor did not indicate the relationship between the location in the chamber of the sampling port for the collecting impinger and the exposure ports. However, it was stated that chamber distribution studies verified that exposure concentrations varied less than 15% between sampling and animal exposure ports, but no data were provided to support this statement.

It is difficult to accept that there is such a difference between whole-body and nose-only exposure that exposure by the latter method at a 30-fold concentration and 6-fold duration compared to the former method, is not as effective as the former in affecting cholinesterase activity of exposed animals.

For rapidity of absorption of soluble substances the lung is second only to intravenous administration, for the lung area is large and the blood flow is high. For some materials, inhalation exposure can be essentially paramount to intravenous administration, which is usually considered to be more effective than either oral or dermal routes. Consequently, any comparisons the sponsor may provide in regard to the total effective dose received by the animals under whole-body and nose-only inhalation exposures to explain the observed differences are solicited. Also, if the sponsor has any information on accomodation, or the lack thereof, to the cholinesterase depressant effects of chlorpyrifos, this could be helpful.

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