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PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: **December 7, 1998**

MEMORANDUM

SUBJECT: ***CHLROPYRIFOS - RE-EVALUATION*** - Report of the Hazard Identification Assessment Review Committee.

FROM: Jess Rowland, Executive Secretary
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

THROUGH: Melba Morrow, Acting, Chairman,
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO: Deborah Smegal
Reregistration Branch 3
Health Effects Division (7509C)

PC Code: 059101

On October 29, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee evaluated new toxicity data and the Registrant's rebuttal, the impact on the existing Reference Dose (Rfd) and the assessment of the potential risk to infants and children from exposure to chlorpyrifos as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Committee Members in Attendance

Members present were: Karl Baetcke, William Burnam, Robert Fricke, Karen Hamernik, Sue Makris, Melba Morrow (Acting Chairman), John Redden and Jess Rowland.

Member in absentia: K. Clark Swentzel,

Data was presented by Deborah Smegal of Reregistration Branch 3.

HED staff participating at the meeting were William Sette of Science Analysis Branch and Kathleen Raffaele of Toxicology Branch 2.

Also in attendance were Brenda Tarplee of Science Analysis Branch and Jerry Blondell of Chemistry and Exposure Branch 2.

Report Preparation:

Jess Rowland
Executive Secretary

I. INTRODUCTION

On October 29, 1998, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) evaluated new toxicity studies and the Registrant's rebuttal of August 4, 1998 and their impact on the current Reference Dose (RfD) as well as on the assessment of the potential risk to infants and children as required by the Food Quality Protection Act (FQPA) of 1996.

II. BACKGROUND

The Reference Dose (RfD) for chlorpyrifos was established during the Health Effects Division's RfD/Peer review on **February 21, 1986.** The RfD of 0.003 mg/kg/day was derived from a NOAEL of 0.03 mg/kg/day established in a human volunteer study using an Uncertainty Factor of 10 to account for intra-species variation. This RfD was re-affirmed at the subsequent meetings held on **March 4, 1988, September 8, 1993, May 25, 1995 and November 23, 1995**. On **April 28, 1996**, HED's Toxicology Endpoint Selection Committee (TESC) selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments.

On **December 11, 1997** , HED's Hazard Identification Assessment Review Committee (HIARC) re-assessed the RfD in response to a report (*Proposed Reference Dose (RfD) for Acute and Chronic Exposure to Chlorpyrifos Based on the Criteria Described by the Acute Cholinesterase Risk Assessment Task Force and the Available Animal and Human Data)* submitted by the Registrant (MRID No. 44271001). At this meeting, the HIARC also re-assessed the doses and endpoints selected for dietary and non-dietary exposure risk assessments by TESC, and addressed the potential risk to infants and children (as required by FQPA). The HIARC's conclusions are presented in the HIARC report of February 2, 1998 (*Memorandum*: J. Rowland, to B. Madden; HED Document 012471).

The HIARC convened on **May 12-14, 1998** to conduct a comprehensive review of the toxicity data, with special emphasis on the neuro-, developmental and reproductive toxicity data of 40 organophosphates, including chlorpyrifos. Based on hazard assessment alone, the HIARC recommended that the 10x Safety Factor for the protection of infants and children be retained for chlorpyrifos (*Hazard Assessment of the Organophosphates: Report of the Hazard Identification Assesment Review Committee*, dated July, 7, 1998).

HED's FQPA Safety Factor Committee met on **June 15 and 16, 1998** to evaluate the hazard and exposure data for chlorpyrifos and recommended retention of the 10x Safety Factor (as required by Food Quality Protection Act of August 3, 1996) to ensure the protection of infants and children from exposure to these pesticides (A Combined Report of the Hazard Identification Assessment Review Committee and the FQPA Safety Factor Committee, dated August 6, 1998).

On October 29, 1998 the HIARC evaluated the five toxicity studies listed below, the Registrant's rebuttal (of 8/4/98) and their impact on the RfD and FQPA assessment. The executive summaries are presented below.

1. Developmental Neurotoxicity Study - Rat (§83-6)	MRID No. 44556901 & 44661001
2. Cholinesterase and Metabolite Determination -Rat (Non -Guideline)	MRID No. 44648102
3. Special Neurotoxic Esterase Assay (Non-Guideline)	MRID No. 44273901
4. Cognitive Neurotoxicity - Rat (Non-Guideline)	MRID No. 44020901
5. Blood Time Course (Part A) (Non-Guideline)	MRID No. .44648101

III. HIARC's REVIEW OF TOXICITY STUDIES AND OTHER DATA

1. Developmental Neurotoxicity Study - Rat (MRID No. 44556901 & 44661001)

In this developmental neurotoxicity study (MRID 44556901), 25 pregnant Sprague-Dawley rats/group were administered chlorpyrifos (99.8% a.i.) by gavage from gestation day 6 (GD 6) through lactation day 11 at 0, 0.3, 1, or 5 mg/kg/day. An additional 5 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. Dams were examined for body weight, reproductive performance, number of viable pups, and postpartum behavior. During the dosing period, dams were observed daily for signs of autonomic function toxicity. Satellite dams were sacrificed four to five hours post-dosing on GD 20 for ChE analyses to be performed on brain, plasma, and erythrocytes. Offspring were examined for viability at birth, pup/litter survival, body weight, sex ratio, physical development landmarks (eye opening and pinna detachment), observed nursing behavior, and sexual maturation. F_1 generation litters were randomly standardized on lactation day 5 and assigned to 4 subsets for continued observation. On postnatal day (PND) 12, fixed brain weight measurements (10 pups/sex/dose) and neuropathological evaluations including morphometrics (6 pups/sex/dose) were performed on Subset 1 pups, with the remaining 10 pups/sex/dose necropsied for gross lesions. In Subset 2, 8 pups/sex/dose were selected for evaluation of learning and memory; evaluations were performed on PNDs 23-25 and 62-92. These Subset 2 animals were sacrificed on PNDs 97-101, following the last evaluation. The Subset 2 pups not selected for evaluation were necropsied for gross lesions on PND 22. The Subset 3 pups were tested for motor activity on PNDs 14, 18, 22, and 61 and auditory startle habituation on PNDs 23 and 62; all Subset 3 animals were sacrificed on PNDs 63 or 64 following the last evaluation. In Subset 4 pups, fixed brain weights were determined in 10 pups/sex/dose, neuropathological examinations were performed on 6 pups/sex/dose, and all remaining Subset 4 animals (10/sex/dose) were necropsied for gross lesions upon sacrifice on PND 66-77.

Maternal toxicity in the high-dose (5 mg/kg/day) animals was manifested as increased signs of autonomic function toxicity, apparent at the end of gestation as fasciculations (6/25 treated vs 0/25 controls), and during early lactation (days 1-5) as fasciculations (16/24 treated vs 0/25 controls), hyperpnea (8/24 treated vs 0/25 controls), and hyperreactivity (17/24 treated vs 2/25 controls). Dams with all pups dying were increased in the high-dose group (3/23 treated vs 0/25 controls). There were no significant effects on bodyweight, food consumption, or pregnancy parameters. There were no unscheduled deaths in the maternal animals.

Brain ChE activity was decreased in the high-dose (↓90%) and mid-dose (↓18%) dams as compared to control. Erythrocyte (↓41-99%) and plasma (↓43-92%) ChE activities were decreased in a dose-dependent manner in all treated groups.

The maternal toxicity NOAEL was not observed.
The maternal LOAEL was < 0.3 mg/kg/day, based on plasma and RBC cholinesterase inhibition.

For the F_1 generation pups, the high-dose group bodyweights were significantly reduced (↓8-15%) at PND 1 and 5 (pre-and post-culling). Bodyweights were also reduced from birth to PND 22 in Subset 4 high-dose animals (↓5-19%); bodyweight gains were reduced in these animals during the same period (↓5-30%). Additionally, compared to the controls, reduced terminal body weights were observed in the Subset 1 (PND 12) high-dose animals (↓17-19%) and the Subset 4 (PND 66) high-dose males (↓10%). For the F_1 generation adults, body weights of the high-dose males were decreased at PND 22 through 66 (↓11-17% vs controls). High-dose F_1 adult females also weighed less than controls at PND 22 (↓17% vs controls), but were of similar weight at PND 66. Bodyweight gains were also decreased in the high-dose males for the PND 22-40 interval (↓13% vs controls) and PND 40-66 interval (↓7%). Food consumption was decreased immediately after weaning (PND 23-30) in high-dose males and females (↓13% vs controls).

Development as assessed by pinna unfolding was delayed (4.0 days in treated vs 3.5 days in controls) in the high-dose group. Sexual maturation was delayed as assessed by time to preputial separation (106% of controls) and vaginal patency (103% of controls).

Pup viability was reduced as assessed by the following parameters: surviving pups/ litter (↓27%) and live litter size at culling (↓16%), pup viability index (↓29%), and pups found dead or presumed cannibalized (day 1 -7.2 % treated vs 0.0% controls; days 2 to 5 -24.7% treated vs 1.3% controls).

There were no statistically significant differences between the groups in the average acquisition and delay training. Additionally, there were no differences among dose groups when comparing retention of information during PNDs 23-25 and 62-92. Motor activity was decreased in high dose male and female pups on PND 14 (↓56% in males and ↓37% in females), and increased in high dose females on PNDs 18 and 22 (↑51% on both days). There was a statistically significant increase (↑16-25%) in the latency to peak response during the auditory startle habituation assessments on PND 23 in the high-dose animals compared to concurrent controls. At PND 62, the latency to peak response in the high-dose animals was 10-12% higher than in the controls. Additionally, the peak response amplitudes in the high-dose animals were decreased by 9 to 29% on PNDs 23 and 62 (not statistically significant) compared to the controls.

There were no gross or microscopic lesions of the nervous system in Subset 1 or 4 offspring. Subset 1 high-dose males at PND 12 had reduced absolute brain weights (↓9% vs controls), increased relative brain weights (↑13% vs controls), reduced anterior to posterior measurement of the cerebellum (↓24% vs controls), reduced height of the cerebellum (↓14% vs controls), decreased thickness of the parietal cortex (↓6% vs controls), and decreased thickness of the hippocampal gyrus (↓9% vs controls). High-dose female pups had reduced absolute brain weights (↓9% vs controls), increased relative brain weights (↑14% vs controls), thickness of the parietal cortex (↓6% vs controls), width of the caudate-putamen (↓10% vs controls), and thickness of the hippocampal gyrus (↓12% vs controls). In Subset 4 F1 animals, killed on PND 66, morphometric analysis revealed decreased parietal cortex measurements (↓5%) and decreased thickness of the hippocampal gyrus

(↓7%) in high-dose females. These measurements were also decreased in mid-dose females (parietal cortex, ↓4%; hippocampal gyrus, ↓4%). The statistical significance of the differences in mid-dose females was not evaluated, and there was no evaluation of low dose females. Brain weight in high dose females was similar to control brain weight at day 66 (↓0.3%).

Due to inadequate presentation of the statistical data analysis, it was not possible to determine the definitive developmental neurotoxicity NOAEL and LOAEL for the offspring. **The tentative developmental neurotoxicity LOAEL is 5 mg/kg/day. The tentative NOAEL is 1 mg/kg/day.**

This study in the rat is classified **unacceptable (§83-6)** and does not satisfy the guideline requirements for a developmental neurotoxicity study. The study may be upgradable, following submission of more complete statistical analysis.

The HIARC also concluded that quantitatively (i.e., based on NOAELs/LOAELs in dams vs. pups), there was no evidence of increased susceptibility. Qualitatively, there was evidence of increased susceptibility at the high dose (5 mg/kg/day) based on the concern for the severity of effects seen in the dams and pups. Maternal toxicity, manifested as increased signs of autonomic function toxicity was apparent at the end of gestation as fasciculations, and during lactation as fasciculations, hyperpnea, and hyperactivity. Offspring toxicity manifested as decreases in body weight/body weight gain and food consumption in both sexes, reductions in pup viability, delays in development (pinna unfolding and sexual maturation), decreased brain weight, and alterations in morphometric measurements. Effects at the mid-dose (1.0 mg/kg/day) are not yet fully determined.

2. Cholinesterase and Metabolite Determination - Rat (Non-Guideline)(MRID No. 44648102)

This study complements a Developmental Neurotoxicity study and was designed to evaluate cholinesterase inhibition and determination of chlorpyrifos and its principal metabolites in dams and pups. Pregnant Sprague-Dawley CD® rats were administered chlorpyrifos (99.8% a.i.; Lot No. MM930503-17; TSN 100227) by gavage at doses of 0, 0.3, 1.0, or 5.0 mg/kg/day beginning on gestation day (GD) 6 and continuing through lactation day 10. Five dams, as well as 5 male and 5 female pups/dose, were sacrificed on GD 20 and lactation days 1, 5, and 11 for chlorpyrifos and metabolite determinations. Milk samples were taken from the dams for chlorpyrifos and chlorpyrifos-oxon analyses. Blood samples were taken from dams and pups for chlorpyrifos, chlorpyrifos-oxon, and 3,5,6-trichloro-2-pyridinol (TCP) analyses. Cholinesterase (ChE) was determined in an additional 5 dams/dose and 5 pups/sex/dose on GD 20 and lactation days 1, 5, 11, 22, and 65 (pups only). ChE activity was determined in plasma, RBC, brain, and heart. For all analyses, samples were taken from dams and fetuses 4 hours postdosing, and from pups 2 hours postdosing of the dams.

No treatment-related clinical signs of toxicity in dams or pups and no differences in maternal body weights were observed at any time during the study. No differences in litter sizes at parturition or in the number of pups born dead were observed between the treated and control groups. Pup survival during lactation was similar for the treated groups as compared to the control.

Chlorpyrifos was detected in the blood of high-dose dams at a mean concentration of 108.78 ng/g on GD 20. Levels of chlorpyrifos then declined to 87% on lactation day 1, remained unchanged on lactation day 5, and were below the limit of detection by lactation day 11. Chlorpyrifos was detected at a low level (2.55 ng/g) in blood of mid-dose dams only on GD 20 and was not detected at any time in blood of low-dose dams. In milk, chlorpyrifos concentrations in the 0.3, 1.0, and 5.0 mg/kg/day groups were 20.57, 139.49, and 3022.00 ng/g, respectively on lactation day 1 and were 13.54, 81.76, and 1533.98 ng/g, respectively on lactation day 5. By lactation day 11, chlorpyrifos was detected only in the high-dose group at a level of 19.79 ng/g. Chlorpyrifos-oxon was not detected in the blood or milk of any dams at any time point.

Blood concentrations of chlorpyrifos in male and female fetuses from high-dose dams were 52.81 and 39.40 ng/g, respectively on GD 20. Concentrations in the pups declined to less than half of the GD 20 levels by lactation day 1 and were below the limit of detection by lactation day 5. Levels of chlorpyrifos in the blood of male and female fetuses from the mid-dose dams were 0.99 and 1.19 ng/g, respectively on GD 20, but were undetectable thereafter. Chlorpyrifos-oxon was detected in the blood of male and female fetuses from high-dose dams only on GD 20 at concentrations of 0.97 and 0.94 ng/g, respectively.

TCP was detected in the blood of dams from all treated groups on GD 20, lactation day 1, and lactation day 5. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood were 114.40, 322.01, and 1974.00 ng/g, respectively on GD 20, and were 142.93, 536.53, and 1449.92 ng/g, respectively on lactation day 5. On lactation day 11, TCP was detected only in the mid- and high-dose groups at levels of 9.87 and 71.40 ng/g, respectively.

TCP was detected in the blood of male and female fetuses from all dose groups in a dose dependent pattern on GD 20. In the 0.3, 1.0, and 5.0 mg/kg/day groups, TCP levels in blood on GD 20 were 93.93, 361.00, and 1680.00 ng/g, respectively for males, and were 99.49, 339.13, and 1884.00 ng/g, respectively for females. TCP was essentially not detectable in the blood of low- and mid-dose pups by lactation day 5. On lactation day 11, TCP was detected in the high-dose male and female pups at levels of 42.29 and 47.01 ng/g, respectively.

ChE activity in fore- and hindbrain from high-dose dams was 11.1-22.7% and 19.5-42.8%, respectively of the control level on GD 20 through lactation day 11 and 57.9% and 80.4%, respectively of controls on lactation day 22. ChE activity in the heart of high-dose dams was 16.9% of controls on GD 20, but recovered to 93.6% of controls on lactation day 22. Mid-dose dams had inhibition of brain ChE activity to 87.8-93.1% of controls from GD 20 through lactation day 11. In low-dose dams, brain and heart ChE activities were unaffected by treatment. In high-dose dams plasma and RBC ChE activities relative to the controls were 12.0% and 4.9%, respectively on GD 20, and 48.9% and 7.4%, respectively on lactation day 11. By lactation day 22, plasma ChE had recovered but RBC activity remained inhibited at 53.6% of the control level. In mid-dose dams plasma and RBC ChE activities relative to the controls were 38.5% and 17.6%, respectively on GD 20, and 65.5% and 22.3%, respectively on lactation day 11. By lactation day 22, plasma ChE had recovered but RBC activity remained inhibited at 66.6% of the control level. In the low-dose group, plasma and RBC activities were 67.2% and 73.7%, respectively of controls on GD 20, but recovered to 83.8% and 75.5%, respectively on lactation day 11 and were similar to controls on lactation day 22.

No effects on ChE activity were seen in tissues from pups from the low- or mid-dose dams. In pups from the high-dose group, forebrain activity was 40.2% of controls on GD 20 and 63.3% on lactation day 1; hindbrain activity was 46.1%, 67.2%, and 88.4% of control levels on GD 20, lactation day 1, and lactation day 5, respectively. ChE activity in the heart of high-dose pups was 18.4%, 34.7%, and 83.9% of control levels on GD 20, lactation day 1, and lactation day 5, respectively. ChE activity in plasma from the high-dose pups was inhibited to 15.3% of controls on GD 20, 40.0% of controls on lactation day 1, and 81.5% of controls on lactation day 5. ChE activity in RBC from the high-dose pups was inhibited to 7.9% of controls on GD 20, 14.7% of controls on lactation day 1, and 86.4% of controls on lactation day 11. Complete recovery of ChE activity occurred in high-dose pups by lactation day 5 for forebrain, by lactation day 11 for hindbrain, heart, and plasma, and by lactation day 22 for RBC.

This study is classified as **Acceptable-nonguideline**. This is a special study intended to investigate specific parameters and does not fit into a guideline study classification. It is acceptable for the purposes for which it was intended. No NOAELs or LOAELs could be determined from the data, because this is a pharmacokinetic study.

The HIARC concurred with the results of this study which, although limited in scope, did not identify any increased sensitivity of neonatal rats (as compared to adults) to chlorpyrifos exposure. This study demonstrated the presence of chlorpyrifos in the blood of dams and fetuses; and the presence of chlorpyrifos in the milk at higher levels than the blood. The HIARC suggested that this study identifies the following NOAELs/LOAELs for cholinesterase inhibition: In pups, the NOAEL and LOAEL are 1 and 5 mg/kg/day, respectively for plasma and red blood cell cholinesterase inhibition, while in dams the LOAEL is $\leq$ 0.3 mg/kg/day (the lowest dose tested) and a NOAEL was not established.

3. Special Neurotoxic Esterase Assay (Non-Guideline) (MRID No. 44273901)

In a special study designed primarily to assess for the potential of chlorpyrifos to inhibit neurotoxic esterase (NTE), chlorpyrifos was administered by gavage to six groups of Fischer 344 strain female rats at dose levels of 0, 1, 5, 10, 50 or 100 mg/kg and sacrificed 24 hours later. NTE was assessed for by the method of Kayyali et al (J. Anal. Toxicol. 15:86-89 (1991). Dosing was by gavage at a dosing volume of 10 ml/kg. The rats were also assessed for cholinesterase inhibition in the plasma, red blood cells (RBCs), heart and brain and there was an additional group dosed at 0.5 mg/kg included for assessment of cholinesterase only. The cholinesterase inhibition data indicated a NOAEL and LOAEL for plasma cholinesterase (ChE) and RBC and heart acethycholinesterase (AChE) of 1 and 5 mg/kg, respectively. At 10 mg/kg, plasma ChE, RBC AChE and heart AChE were inhibited approximately 45%, 17% and 19%, respectively. Brain AChE demonstrated a NOAEL and LOAEL of 10 and 50 mg/kg, respectively and at 50 mg/kg inhibition was approximately 53%. NTE was not inhibited at the highest dose level of 100 mg/kg and there was an apparent 9% increase in activity at this dose level.

This study is classified as **Acceptable (non-guideline).** This study contains data useful for evaluating the potential for chlorpyrifos to inhibit neurotoxic esterase following systemic administration.

The HIARC concurred with the results of this study which demonstrated a NOAEL of 1 mg/kg/day and a LOAEL of 5 mg/kg/day for inhibition of plasma and red blood cell cholinesterase activity; a NOAEL of 10 mg/kg/day and a LOAEL of 50 mg/kg/day for inhibition of brain cholinesterase activity; and no inhibition of neurotoxic esterase at the highest dose tested (100 mg/kg/day).

4. Cognitive Neurotoxicity Study - Rats (Non-Guideline) (MRID No. 44020901)

In this special study (MRID 44020901) that is *tentatively* classified as unacceptable but upgradeable because additional data are being requested, the effects of repeated oral administration of chlorpyrifos technical (purity, 98.1%; lot no. #MM-890115-616) on the cognitive function of rats were evaluated with a delayed matching to position (DMTP) test. Groups of 10 *female* Long-Evans rats, pretrained in a DMTP apparatus were administered oral doses of chlorpyrifos in corn oil of 0, 1, 3, or 10 mg/kg/day for 5 days/week for 4 weeks. DMTP testing was conducted 6 days/week during treatment and continued post-dosing for 4 weeks. Testing for short-term memory (as evidenced by the retention rate) and attention/encoding deficits was based on the percent correct accuracy on several time delays. Slope over delay and intercept at time zero were calculated from these data for each rat and represented the "forgetting curve." A satellite group of 6 rats/dose was sacrificed after the 4-week dosing period and plasma, erythrocyte and brain cholinesterase (ChE) were determined. Neurotoxic esterase (NTE) activity was determined in satellite rats from the control and high-dose groups.

ChE and (NTE) were assessed one day after the last dose administration. Plasma (68%), RBC (56%) and brain (8%) ChE were inhibited at 1 mg/kg/day. At 3 mg/kg/day, plasma (83%) RBC (65% and brain (63%) inhibition increased. At 10 mg/kg further increases were noted. NTE was not affected (6% decrease in the high-dose group was not considered significant). **The LOAEL for ChE inhibition is < 1 mg/kg/day. No NOAEL was established.**

The clinical sign of **miosis** was observed in rats that received 3 and 10 mg/kg/day particularly at weeks 3 and 4 and corresponded with brain ChE inhibitions of 63% and 86%, respectively. **Salivation** and **tremors** were observed primarily in the high-dose group (10 mg/kg/day; brain ChE inhibition of 86%) with tremors usually disappearing by the following morning. The **DMTP parameters** of actual total delay (increased), void trials per session (increased) and nosepokes (decreased) were affected by 10 mg/kg/day chlorpyrifos at most or all intervals during dosing. The **intercept of the retention gradient** (an index of the attention/encoding processes) was increased at week 2 and decreased at week 3 but equivalent to the control at weeks 1 and 4 at 10 mg/kg/day. The possibility that there is an effect on the intercept of the retention gradient requires further analysis. In order for HED to complete its assessment of chlorpyrifos on the cognitive ability of the rat and further evaluate the possible relationship between the effects on DMTP performance and cognitive ability, additional data tables on the percent correct responses at all assay intervals are being requested. **The *tentative* conclusion is that the study supports a LOAEL for neurotoxic systemic effects of 3 mg/kg/day based on miosis. The NOAEL for neurotoxic systemic effects is 1 mg/kg/day. A tentative LOAEL on the DMTP performance is 10 mg/kg/day. Conclusions related to possible effects of chlorpyrifos on the cognitive ability of the rat are *pending*.**

This study is ***tentatively*** classified **Unacceptable** (Nonguideline) ***but upgradeable***. Additional data on summary tables on the percent correct responses of each delay, the key measure made to assess for cognitive effects in this study are being requested in order to complete the review of this study. The percent correct responses should also be analyzed statistically by the laboratory.

The HIARC concluded that the dose levels tested in this study were higher than those which caused cholinesterase inhibition in other studies. The HIARC further concluded that the results of this study provided data on the cognitive functions of the rats following repeated exposure which are essential for hazard characterization and qualitative assessment of the overall toxicity of chlorpyrifos but which will not have an impact on risk assessment. The HIARC also concluded that the data presented need additional analysis, specifically, the study needs to present summary tables of the percent correct at a function of delay.

Therefore, the HIARC recommended that this study should be classified as **Unacceptable** pending receipt and review of the additional data requested by this Committee, and the Data Evaluation Report should reflect this conclusion.

5. Blood Time Course (Part A) (Non-Guideline) (MRID No. 44648101)

This study was done to help construct and validate a physiologically-based pharmacokinetic model for chlorpyrifos (Unlabeled -99.8% a.i., Lot # MM930503-17; Labeled -89.4% a.i., Lot # B930-51 [INV1134]) a weak inhibitor of acetylcholinesterase activity, and its metabolites, chlorpyrifos-oxon (OXON), a strong cholinesterase inhibitor and 3,5,6-trichloropyridinol. Groups of 24 male rats were given a single gavage dose of 0.5, 1, 5, 10, 50, or 100 mg/kg chlorpyrifos in corn oil. Four rats from each group were killed 10 and 20 minutes and 1, 3, 6, and 12 hours after treatment. Cholinesterase activity was measured in the brain and plasma at each time point, as well as the plasma concentration of the test material and its OXON metabolite. In a separate portion of the study, four male rats were given a single gavage dose of labeled chlorpyrifos at a concentration of 5 or 100.0 mg/kg and were sacrificed three hours later. Blood was collected from the animals at sacrifice and the concentration of the test material and its metabolites 3,5,6-trichloropyridinol (TCP) and OXON determined. Plasma cholinesterase activity decreased in a time-and dose-dependent manner. The plasma cholinesterase activities of rats treated with 0.5, 1, 5 or 10 mg/kg were maximally decreased 3-6 hours after treatment, with both the decrease and recovery of activity being dose-dependent. The decrease in activity of rats treated with 50 or 100 mg/kg began within 10 minutes of treatment. By 12 hours after treatment, both groups were approximately 11% of the control group and had not shown signs of recovery.

Brain cholinesterase activity was not affected as dramatically by test material treatment as plasma activity with only the 10, 50, and 100 mg/kg dose groups showing significant effects. The brain cholinesterase activity of rats treated with 10 mg/kg test material began to decline within three hours of treatment and was significantly decreased by six hours after treatment. The brain cholinesterase activity in the 50 or 100 mg/kg dose groups decreased significantly within one hour of treatment; and by 12 hours, were approximately 30% and 20%, respectively, of control. In none of the affected groups did brain cholinesterase show signs of recovery. Peak chlorpyrifos blood concentrations occurred within three hours of treatment in all but the lowest dose group. The area under the curve

(AUC) was calculated as 0.4, 1.1, 5.0, and 12.5 μmole hr L^{-1} for the 5.0, 10.0, 50.0, and 100 mg/kg groups, respectively and yielded calculated blood half-lives of chlorpyrifos of 2.7,1.5, 2.1, and 7.3 hours for the 5.0, 10.0, 50.0, and 100.0 mg/kg dose groups, respectively. Regardless of dose, the highest concentration of OXON detected was 2.5 ng/g found in the blood of rats treated with 50 mg/kg test material one hour post-treatment. Following treatment with 5 or 100 mg/kg labeled test material, 98% of the activity detected in the blood was identified as TCP metabolite with the remaining attributed to the parent compound. Since OXON is an intermediate in the formation of TCP and none of the metabolite was detected, these studies support that the half-life of the OXON metabolite is short (reportedly 10 seconds) and that *in vivo* metabolism of chlorpyrifos is rapid.

This study is considered **Acceptable (nonguideline).** It may partially fulfill guideline requirements in other areas.

6. Review of Registrants Letter to the Agency (August 4, 1998).

The Registrant's letter provides review of and makes comment on a variety of studies submitted to OPP/HED or published in the open literature. Except for the results of the developmental neurotoxicity study which was reviewed at this meeting, no new data or analyses were provided in this letter. All other materials had been previously considered by HED's RfD/Peer Review Committee or in earlier meetings of HIARC. The RfD Peer Review Report of 2/26/96 and the HIARC report of 2/2/98 discussed the same data from the Registrant in terms of acute and repeated animals and human exposures.

Again, the HIARC, re-affirmed the use of the human volunteer study (Coulston *et al.* 1972) and the NOAELs used in deriving the acute and chronic RfDs.

The Agency selected the NOAEL of 0.1 mg/kg (established in the Coulston *et al,* 1972 study) for deriving the **Acute RfD** because no effects were seen on plasma cholinesterase activity after 1 and 3 days of treatment but significant inhibition of plasma cholinesterase activity was seen at this dose (0.1 mg/kg/day) after only 6 and 9 days of treatment. Therefore this dose is appropriate for acute (single exposure) dietary risk assessment.

The Registrant, however, requested the use of 0.5 mg/kg for the NOAEL based on another human study (Nolan *et al* 1982). In the Agency's presentation to the FIFRA Scientific Advisory Panel (SAP) in June 1997, it was noted that on Day 1 of this study, plasma cholinesterase activity was inhibited 84 to 86% while by Day 4, red blood cell acetylcholinesterase activity was inhibited by 11 to 52%. Therefore, the HIARC rejected the 0.5 mg/kg as a NOAEL for either plasma or red blood cell cholinesterase inhibition from the Nolan study.

The Agency selected the NOAEL of 0.03 mg/kg/day (established in the Coulson *et al* , 1972 study) for deriving the **Chronic RfD** based on significant inhibition of plasma cholinesterase activity (reduced 18-64% from pre-test levels) and clinical signs (blurred vision, runny nose and faintness in 1/4 subjects) at 0.1 mg/kg/day (LOAEL). Treatment of subjects at this dose (0.1 mg/kg/day) was terminated at 9 days because plasma cholinesterase inhibition exceeded 20-30%, which the study authors used as a guideline.

The HIARC again contends that there is no reason to discount the observed clinical signs, because blurred vision is a typical cholinergic sign of cholinesterase inhibition and can not be attributed to a common cold, as purported by the Registrant. In addition, there is no reason to believe that other clinical signs would not have appeared if the dosing had continued for 20 days as it did for the other groups.

The Registrant recommends a chronic RfD of 0.01 mg/kg/day based on a NOAEL 1 mg/kg/day for brain acetycholinesterase inhibition in animals. The HIARC noted that several animal studies identify NOAELs less than 1 mg/kg/day as discussed below.

The HIARC noted that at least eight animal studies in rats, mice and dogs provide support for lower chronic RfDs. Overall, these studies identify LOAELs for plasma, red blood cell and brain cholinesterase inhibition in the range of 0.2 to 1 mg/kg/day following exposures of 10 days to 2 years (Barker 1989, Crown 1990, Deacon *et al.* 1979, Hoberman 1998a,b, Kociba 1985, McCollister *et al.* 1971, Rubin *et al.* 1987a, Szabo *et al.* 1988, Young and Grandjean 1988). Most noteworthy is the chronic rat study that observed significant inhibition of brain cholinesterase at 1 mg/kg/day in both sexes (Young and Grandjean 1988). Collectively, these studies identify NOAELs in the range of 0.01 to 0.1 mg/kg/day. In addition, the developmental study by Ouellette *et al.* (1983) also supports a NOAEL of 0.1 mg/kg/day, but observed plasma and red blood cell cholinesterase inhibition at higher levels of 3 mg/kg/day (only tested 0.1, 3 and 15 mg/kg/day).

If these animal data were selected as the basis of the chronic RfD, and an Uncertainty Factor of 100 was applied to account for intra-specis variability (10x) and inter-species extrapolation (10x), this would yield much lower chronic RfDs (range, 0.0001 to 0.001 mg/kg/day) than the current RfD of (0.003 mg/kg/day) derived from the human data with a UF of 10x for intra-specis extrapolation.

The HIARC, after careful consideration of all available animal and human data and the proposals put forth by the Registrant, concluded that there is no reason for changing the doses, endpoints, and/or the uncertainty factors selected for deriving the acute and chronic RfD's for dietary as well as occupational/residential exposure risk assessments.

7. An Overview of Poisoning Incident Data

An overview of the poisoning incident data is presented here. A detailed evaluation is presented in Appendix 1.

As a result of the widespread use of chlorpyrifos, there have been numerous exposures and poisonings. Detailed analysis of the poisoning data has been used to identify specific use patterns that are more likely to be associated with pesticide poisoning. In addition to acute poisoning, chlorpyrifos has been reported to be associated with chronic effects in humans, including peripheral neuropathy, chronic neurobehavioral effects, and multiple chemical sensitivity.

The main source of serious, acute incidents of chlorpyrifos poisoning appears to be liquids (not including aerosol cans) used by homeowners or Pest Control Operators (PCOs) indoors or outdoors, termite treatments, and liquid sprays and dips applied to domestic animals. Poisonings are likely to be more serious when applied by a PCO (usually due to misuse). However, in 1998 the uses of sprays and shampoos on pets, broadcast uses on carpets, paint additives, and indoor fogger use were voluntarily canceled. However, even when these canceled uses are excluded, it appears that poisonings are likely to be more serious when applied by a PCO (usually due to misuse). This is supported by reports received by Dow AgroSciences, the American Association of Poison Control Centers, the California Pesticide Illness Surveillance Program, and the Incident Data System of the Office of Pesticide Programs (OPP).

IV. DETERMINATION OF INCREASED SUSCEPTIBILITY

The developmental neurotoxicity study demonstrated that quantitatively, based on the NOAELs and LOAELs, there is no evidence of increased susceptibility in neonatal rats when compared to adult rats. The NOAEL (1 mg/kg/day) in the F_1 offspring for developmental neurotoxicity was higher than the maternal toxicity (no NOAEL). However, qualitatively the effects in the F_1 offspring at the high dose were more severe when compared to those observed in the dams. Specifically, at a dose which resulted in cholinesterase inhibition and transient cholinergic clinical signs in the dams, observations in the offspring included postnatal death; decreased body weight, body weight gain and food consumption; delayed development (pinna unfolding and sexual maturation); decreased brain weight; and alterations in post natal day 11 morphometric measurements, some of which persisted through to adulthood (postnatal day 60). It should be noted that the parameters evaluated in the offspring and the dams in this study protocol are not comparable. Nevertheless, in a subchronic neurotoxicity study in rats, no neurobehavioral or neuropathological effects were observed in adult animals at the highest dose tested of 15 mg/kg/day (MRID 42929801) which support the argument that the offspring appear to be more severely affected by chlorpyrifos exposure than are adult animals.

At the December 11, 1997 meeting, in the absence of the developmental neurotoxicity study, the HIARC concluded that a review of the data submitted to the Agency as well as numerous published and unpublished research papers provided sufficient information to elicit concern about the potential for increased susceptibility of neonates to chlorpyrifos exposure. On the other hand, there was no indication of increased susceptibility of fetuses to chlorpyrifos following *in utero* exposure. The studies from which these conclusions were derived are presented in the Committee report dated 2/2/98 (HED Document No. 012471).

At the10/29/98 meeting, the HIARC determined that there is sufficient evidence to conclude that exposure to chlorpyrifos results in increased susceptibility to neonates as compared to adult rats. This conclusion is based on the evidence presented in the guideline developmental neurotoxicity study submitted to the Agency and also of the published studies (some of which are described below) which provide evidence that the perinatal rats are more sensitive to chlorpyrifos than dams.

Moser and Padilla (1998) reported that following oral (gavage) administration of 75 to 80% of the maximum tolerated dose, neonatal rats were 5 times more sensitive than adult rats. Following a single oral dose to pups (postnatal day 17) and adults (about 70 days), although the degree of cholinesterase inhibition (blood, brain and peripheral tissues) and behavioral measurements were similar, the effective dose was five-fold lower for the pups (15 mg/kg/day) when compared to adults (80 mg/kg). In the same study, 10 day old pups were approximately 7 times more sensitive than adults based on the maximum tolerated dose (15 mg/kg vs. 100 mg/kg, respectively).

Lasstier *et al* (1998) observed that following gavage doses of chlorpyrifos at 7 mg/kg on gestation days 14 to 18, the time of maximal cholinesterase inhibition (ChEI) was the same for both maternal and fetal brain, the degree of fetal brain ChEI was 4.7 times less than the maternal brain ChEI. Also, the detoxification potential (i.e., carboxylesterase and chlorpyrifos-oxonase) of the fetal tissue was very comparable to maternal tissues. Following a single dose (7 mg/kg/day) on gestation day 18, the degree of fetal brain ChEI was comparable to the maternal brain ChEI. The maternal brain cholinesterase was inhibited more than the fetal brain cholinesterase (more than 4 fold) only after repeated dosing (gestation days 14 to 18).

Based on these results the authors concluded that the fetus is not genuinely protected from the toxic effects of a given dose of chlorpyrifos but that the fetal brain cholinesterase is simply able to recover more fully between each dose as compared to maternal brain cholinesterase. This gives the illusion that the fetal compartment is less affected than the maternal compartment.

Pope *et al* (1991) reported that following subcutaneous injection, the neonates were more sensitive to chlorpyrifos exposure than adults; the maximum tolerated dose for neonates was 45 mg/kg, while for adults the MTD was 279 mg/kg.

Pope and Liu (1997) reported that following subcutaneous injection when the endpoint was based on 50% acethylcholinesterase inhibition, the neonates were only about two times more sensitive than adult rats; ED_{50} = 19.8 mg/kg for neonates and 44 mg/kg for adults.

Whitney *et al* (1995) administered chlorpyrifos by subcutaneous injections to neonatal rats in apparently subtoxic doses that caused no mortality and little or no weight deficits, and examined developing brain regions (cerebellum, forebrain and brainstem). One-day old rats showed significant inhibition of DNA and protein synthesis in all brain regions within 4 hours of treatment with 2 mg/kg. The authors concluded that chlorpyrifos affects the developing brain during cell division.

Intraperitoneal injection of chlorpyrifos at toxic doses has resulted in blockage of protein and DNA synthesis, modification of the activity of the adenylyl cyclase system, and binding of specific ligands to the beta-adrenergic or muscarinic receptors in the brain of 1 to 5 day old neonatal rats (Whitney *et al.*, 1995, Cambel *et al.*, 1997 and Song *et al.*, 1997).

The Registrant contends that increased susceptibility demonstrated by Moser and Padilla (1998) is not relevant due to the route of administration. The Registrant claims that direct administration of chlorpyrifos by gavage to 17-day old rats is not a natural route of exposure, that the dose was excessive and unrealistically toxic relative to what could be absorbed via milk. In addition, even if it were assumed that direct administration is an appropriate route there is no way to relate the quantity administered by gavage to the amount that might be absorbed through the diet by the pup at this age.

The Registrant also contends that the increased susceptibility seen following subcutaneous and the intraperitoneal routes of administration are not relevant because the impact of the vehicle (DMSO) is indeterminable, only very toxic doses were studies, no effort was made to define a NOAEL, and the route of exposure (i.e, subcutaneous and intraperitoneal) are not relevant for the route of concern (oral) for human risk assessments.

The HIARC acknowledges that while young rats (and by extrapolation, young humans) appear to be more sensitive to the acute toxicity of high doses of chlorpyrifos, sensitivity to neurochemical and/or neurobehavioral changes following repeated, low-dose exposure should be of more concern for risk assessment and regulatory decisions making, especially in light of the FQPA.

The HIARC determined that the results of the oral, subcutaneous and intraperitoneal studies described above and others presented in the previous HIARC report (2/2/98)can not be discounted since there are no studies that evaluate susceptibility or sensitivity in neonatal rats at low doses that simulated potential "real world" exposure to chlorpyrifos. Although no increased susceptibility was seen in the developmental neurotoxicity study at low doses, the experimental design of this study is vastly different from the Moser and Padilla (1998) study in which the neonates were shown to be 5 times more sensitive than adult rats.

The HIARC acknowledges that the subcutaneous and intraperitoneal exposure are not routes which are anticipated for human exposure routes (i.e, oral); however, the results of these studies can not be discounted because they are supportive of the increased susceptibility seen via the oral route and are considered relevant as a part of the weight-of-evidence in assessing age-related differences in susceptibility to chlorpyrifos exposure.

Based on the weight-of-evidence considerations and the lack of data with lower, "real world" exposure to chlorpyrifos in the diet, the HIARC concluded that the current available data demonstrated that exposure to chlorpyrifos results in increased susceptibility of young rats (and by extrapolation young humans) and this evidence can not be discounted.

V. RECOMMENDATION ON THE FQPA SAFETY FACTOR

Based solely on the hazard assessment, the HIARC considered the retention of the 10x Safety Factor for the protection of infants and children (as required by FQPA) for the following reasons:

i. Increased susceptibility was demonstrated in neonatal rats when compared to adult rats following oral, subcutaneous and intraperitoneal routes of exposure in studies published in the open literature.

ii. Although the subcutaneous and intraperitoneal injections do not represent traditional routes of exposure (i.e., oral), the results of these studies support the results observed following oral exposure and thus can not be discounted.

iii. Increased sensitivity of the offspring was noted in the developmental neurotoxicity study in which effects on the offspring at the highest dose tested (5 mg/kg/day) were more severe than effects observed in the dams at the same dose. The relative lack of sensitivity of the dams was confirmed by the results of a subchronic neurotoxicity study in adult rats.

v Although the increased susceptibility in the literature studies was seen only at high doses, there are no comparable studies that examined age-related sensitivity at lower doses. Therefore, there is uncertainty related to the absence of these data.

vi. Although no evidence of increased offspring susceptibility was seen at the lowest dose tested in the developmental neurotoxicity study, the experimental dosing in this study was different from that used in the oral study by Moser and Padilla that did show increased susceptibility.

vii. Chlorpyrifos is a neurotoxicant with evidence of OPIDN in humans (following a suicide attempt) and in animals; there have been cases reported of neurophysiological effects in humans.

viii. Poisoning data showed that compared to other organophosphates, chlorpyrifos was 1.8 times more likely to have effects lasting a week or more and 1.6 times more likely to have effects lasting a month or more. Additionally, with the exception of propetamphos, chlorpyrifos had the highest percentage of persistant effects reported for 13 organophosphates used widely in residential settings

Although the above factors provided reasons for retaining the 10 Safety Factor, the HIARC recommended that the 10x Safety Factor can be reduced because:

i The toxicology database is complete.

ii No increased susceptibility was seen following *in utero* exposure in the prenatal developmental toxicity studies in rats submitted to the Agency under Subdivision F Guidelines.

iii No increased susceptibility was seen following in utero exposure in the prenatal developmental toxicity studies in rabbits submitted to the Agency under Subdivision F Guidelines.

iv Quantitatively, that is, based upon a comparison of maternal and offspring NOAELs, and not considering the severity of the effects, no increased susceptibility was seen following pre/post natal exposure in the two generation reproduction study in rats submitted to the Agency under Subdivision F Guidelines.

v No increased susceptibility was seen in the developmental neurotoxicity study in rats submitted to the Agency under Subdivision F Guidelines.

In reaching this conclusion, 4 members voted for the retention of the 10x Safety Factor while 7 members voted for reducing the 10x Safety Factor. The final decision will be made during risk characterization by the FQPA Safety Factor Committee.

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APPENDIX 1

Human Incident Data

Summary

As a result of the widespread use of chlorpyrifos, there have been numerous exposures and poisonings. Detailed analysis of the poisoning data has been used to identify specific use patterns that are more likely to be associated with pesticide poisoning. In addition to acute poisoning, chlorpyrifos has been reported to be associated with chronic effects in humans, including peripheral neuropathy, chronic neurobehavioral effects, and multiple chemical sensitivity.

The main source of serious, acute incidents of chlorpyrifos poisoning had been liquids (not including aerosol cans) used by homeowners or Pest Control Operators (PCOs) indoors or outdoors, termite treatments, and liquid sprays and dips applied to domestic animals. However, in 1998 the uses of sprays and shampoos on pets, broadcast uses on carpets, paint additives, and indoor fogger use were voluntarily canceled. However, even when these canceled uses are excluded, it appears that poisonings are likely to be more serious when applied by a PCO (usually due to misuse). This is supported by reports received by the American Association of Poison Control Centers, the California Pesticide Illness Surveillance Program, and the Incident Data System of the Office of Pesticide Programs (OPP).

American Association of Poison Control Center Database

Of the 116,225 unintentional pesticide exposures to single products in 1996, 19,033 or 16% were due to organophosphate pesticides and 5,188 or 4.5% were due to chlorpyrifos. Given that 30% of organophosphates were not specifically identified by active ingredient, **the actual number of chlorpyrifos cases reported to AAPCC is probably close to 7,000 or 6% of all the pesticide-related exposures. Many of these exposures involve small children who are exposed but never develop symptoms. Increased use of child-resistant packaging could markedly reduce these exposures. Of the cases receiving follow-up, a minority experienced moderate effects (7.7%), major or life-threatening effects (0.4%), and there was one fatality. The other 92% either developed no symptoms or minor symptoms as a result of their exposure. In 1996 there were 1,109 symptomatic cases reported to Poison Control Centers judged to have effects related to the exposure. From 1993 through 1996, there were an average of 116 chlorpyrifos cases per year with moderate to fatal outcome reported in residential settings.**

Poison Control Center data combined for the years 1993-1996 was examined to determine hazards from organophosphate pesticides used in residential settings. Thirteen organophosphate insecticides were analyzed with at least 100 exposures reported over the four year period. Four measures were selected by HED to assess the amount of hazard associated with chlorpyrifos relative to other insecticides, most of which were used primarily in agriculture. These were: percent of all cases that were seen in a health care facility; percent of cases seen in health care facility requiring hospitalization; and of those case receiving follow-up to determine outcome, percent with symptoms and percent with life-threatening symptoms.

Chlorpyrifos ranked third (out of 13) for symptoms and first for major/fatal outcome reported in adults. Chlorpyrifos ranked third for ratio of symptoms per containers reported in US homes for both children and adults. Chlorpyrifos ranked third for serious outcomes from environmental residues. Environmental residues accounted for 15% of the chlorpyrifos exposures and 30% of the cases with serious outcome (twice as much as reported for non-organophosphates). Chlorpyrifos ranked first for effects persisting longer than a week (more than twice as likely as non-organophosphates) and second for effects lasting longer than a month (nearly three times as likely as non-organophosphates).

This finding is consistent with an earlier review that suggested that chlorpyrifos may be a cause of chronic neurobehavioral effects in some subset of sensitive people who have been poisoned by this compound (Blondell and Dobozy 1997).

For most measures, Chlorpyrifos did not pose a greater hazard than other organophosphates used in residential settings. However, organophosphates as a group pose a greater hazard, especially to young children under six years of age, than other pesticides. Children under six were three times more likely to be hospitalized, five times more likely to be admitted for critical care (ICU), and three times more likely to have experienced a life-threatening outcome or death when exposed to a chlorpyrifos when compared to other non-organophosphates. Adults were 44% more likely to be hospitalized and 40% more likely to be admitted to an ICU if exposed to an organophosphate. Their likelihood of getting symptoms including life-threatening effects was about the same for chlorpyrifos as for other non-organophosphate pesticides.

For both adults and children, the number of symptomatic chlorpyrifos cases per million containers estimated in U.S. homes was about the same as for all organophosphates. Exposure to environmental residues of chlorpyrifos account for 30% of the more serious cases which is twice the proportion reported for non-organophosphate pesticides. Three percent of symptomatic cases report effects lasting longer than a week and one percent report effects lasting longer than a month, substantially more than reported for most other pesticides.

EPA surveyed certified and commercial pesticide applicators in five non-agricultural categories (structural, turf and ornamental, public health, right-of-way, and aquatic) in 1993. A total of 69 million pounds of active ingredient were estimated in use, including 14.4 million pounds of organophosphates (OP) or 20.8% of the total. Over 90% of the OP insecticide use is accounted for by just three active ingredients: chlorpyrifos (54%), malathion (30%), and diazinon (7%).

For the purposes of estimating hazard of PCO use or consumer use, products in the AAPCC database were divided into whether they were likely to be used by PCOs or homeowners. Undoubtedly some misclassification occurred with products in both lists. For example, some of the 'residential' incidents involve agricultural uses not intended for home use. However, the overwhelming majority of incidents were due to products commonly used by homeowners and therefore the small number of incidents involved in misclassification are not expected to unduly influence the results. Table 1 reports the number of incidents on which calculations are based for chlorpyrifos.

Table 1. Number of exposures, symptomatic cases (life-threatening/fatal cases listed in parentheses), seen in a health care facility (HCF), or hospitalized (ICU cases in parentheses) for chlorpyrifos products used by Pest Control Operators and homeowners, Poison Control Centers 1993-1996.

Product type/ Age Group	Exposures	Symptomatic (Life-thr.)	HCF	Hospital. (ICU)
PCO/Child	273	53 (2)	56	16 (8)
Non-PCO/Child	7172	604 (4)	622	60 (26)
PCO/Adult	821	272 (2)	185	19 (8)
Non-PCO/Adult	4517	1598 (11)	817	66 (25)

Table 2. PCO compared with non-PCO use of chlorpyrifos by percent residential cases seen in a HCF, hospitalized, ICU, with related symptoms, and with major or fatal medical outcome for children under age six, PCCs 1993-1996.

Pesticide Type	% seen in a HCF	% Hospit-alized/ICU	% with symptoms	% major or fatal
PCO Use	20.5	28.6/14.3	38.1	1.44
Non PCO Use	8.7	11.8/9.6	18.2	.12
Ratio PCO/Non-PCO	2.4	2.4/1.5	2.1	12.0

Table 3. PCO compared with non-PCO use of chlorpyrifos by percent residential cases seen in a HCF, hospitalized, ICU, with related symptoms, and with major or fatal outcome for adults and children six years and older, PCCs 1993-1996.

Pesticide Type	% seen in a HCF	% Hospit-alized/ICU	% with symptoms	% major or fatal
PCO Use	22.5	10.3/4.3	76.2	.56
Non PCO Use	18.1	8.7/3.1	72.8	.50
Ratio PCO/Non-PCO	1.2	1.2/1.4	1.0	1.1

Table 1 shows that 9 percent of chlorpyrifos exposures were due to PCO products, but 21-24% of the life-threatening/fatal cases, hospitalized cases and cases seen in an ICU were due to PCO products. Tables 2 shows a much greater risk for children under six years of age exposed to products used by PCOs and containing chlorpyrifos. Note that the number of cases involving these PCO products is relatively small compared to consumer products. Of the total 7,445 exposures involving children under age six, just 4% were due to products known to be used primarily by PCOs. For adults and children over six, 15% of the 5,338 exposures examined involve PCO products.

Duration of effects was considered for PCO products for chlorpyrifos. There were 26 cases, 24 of which were due to a single PCO product. Of the 149 symptomatic cases from exposure to this product, 7.9% had symptoms persisting more than one week (6 times higher than non-organophosphates) and 2.7% had symptoms persisting longer than one month (20 times higher than non-organophosphates). Interestingly, another similarly formulated chlorpyrifos PCO-product, but without the odoriferous carrier, did not have any symptomatic cases or even an exposure reported to Poison Control Centers from 1993 through 1996. This suggests that the relatively low toxicity, but odoriferous carrier mixed with chlorpyrifos may be a factor leading to long term effects.

There was an average of 274 exposures per year reported for adults and children involving PCO products containing chlorpyrifos. Though the number of exposures is relatively small, the increased risk of serious effects requiring hospitalization and admission for critical care is significant.

California Pesticide Illness Surveillance System

Cases of health effects attributable to exposure to chlorpyrifos used agriculturally, alone or in combination, reported to California Pesticide Illness Surveillance Program from 1982-1992 were reviewed (Edmiston and Maddy 1987). Activity (type of work being performed during exposure), for purposes of this review, was categorized as applicator, residual, mixer/loader, coincident and other (combined categories).

During the years 1982 through 1992, there were 100 cases in which chlorpyrifos was used alone or in combination, but was judged to be responsible for the illness. The following conclusions were drawn from the analysis of these 100 cases:

1) The applicator activity category was most frequently associated with adverse health effects, accounting for 38% of the cases where chlorpyrifos was considered the primary pesticide associated with the illness. Drift was the second largest category with 35% of the incidents. However, half of the drift cases were due to a single incident in an orange grove in 1989. Note that many cases of drift or exposure to residue in field workers may go unreported because of disincentives associated with seeking medical care and lack of physician reporting.

2) Over one-half of all incidents were systemic poisoning involving applicators and those directly exposed to spray drift. This indicates that when formulated for application, chlorpyrifos exposure can lead to poisoning.

3) The data (number of cases, categories most frequently reported) are fairly consistent from year to year, with the exception of the 18 cases due to one drift incident in an orange grove in 1989.

4) Of the 35 cases involving skin, eye, or respiratory effects, 71% were pesticide handlers, either applicators or mixer/loaders.

5) The number of systemic poisoning cases per 1000 applications ranges from 0 to 0.55. This is fairly consistent with the median (0.41) reported for 28 insecticides analyzed as part of the acute worker risk analysis for the years 1982-1989 (Blondell 1994a). Data on usage suggest that only about one-half of the applications were reported prior to 1989, when only commercial and restricted applications had to be reported. The ratio of chlorpyrifos poisoning to number of applications was similar to that of most of the other 28 insecticide alternatives. **HED concludes that limited available data on chlorpyrifos does not demonstrate an excess risk for agricultural handlers or workers relative to other insecticides, but does recommend that its risks be mitigated where practical as part of the overall approach of the Acute Worker Risk Strategy.**

California Chlorpyrifos Illnesses Involving Structural PCOs

A total of 304 incidents received by the California Pesticide Illness Surveillance Program involving exposure to chlorpyrifos applied by Structural Pest Control Operators (SPCO) from 1982 to 1993, inclusively, were reviewed and analyzed. Note that one additional year of data (1993) is provided that was not available for the agriculturally-related cases reported in California. Excluding 1993, there were a total of 273 SPCO-related cases, almost three times as many as reported for agricultural use of chlorpyrifos. As will be seen, this is partly because more people are present during an application by an SPCO than during agricultural use. Note that SPCO cases involving exposure to non-occupational persons (residential rather than business applications) are much less likely to be reported under the California mandatory reporting requirement. Such cases would not be covered by worker's compensation and, the payment incentive for physician reporting does not apply. Therefore, these types of cases are likely greatly under-reported. On the other hand, many of the cases (exact number not known) were due to broadcast carpet or fogger uses which have since been canceled.

The 304 cases were also analyzed by activity category. In 46 of the 304 incidents (15%), there was an indication that an accident occurred which resulted in the exposure, most commonly a hose breaking. Failure to wear safety protection (mostly goggles) or lack of safety training was reported in 21 cases (7%). The comments sections also contain several incidents where pesticide application was made while people were in the premises.

Upgrading requirements for certification and application should be considered for pesticides like chlorpyrifos that can result in damage to property and significant adverse health effects that may cost thousands of dollars per case. Removing bystanders from the immediate site of application and thorough ventilation would prevent a large number of these cases.

Literature Reports on Acute Effects

Hodgson et al. (1986) reported on five office workers poisoned primarily by inhalation exposure to chlorpyrifos. Exposure occurred through an air intake vent on a Friday, 2 of the workers were also present for 8 hours on Saturday and Sunday. All five workers reported symptoms the following Monday. Symptoms and number of individuals reporting them were: chest tightness (3), cough (2), visual symptoms (2), drooling (3), sweating (3), nausea (4), diarrhea (4), abdominal pain (3), weakness (4), fatigue (5), restless (2), anxiety (4), confusion (2), and disturbed speech (1). Measurements of red blood cell cholinesterase levels found that recovery to normal took up to 80 days. Three weeks later one person reported numbness and tingling in the fingertips of both hands which lasted one week. According to Berger and Schaumberg (1994), a case of paresthesia involving only the upper extremities should not be regarded as evidence of toxic neuropathy. Hodgson states that this application was in conformance with label directions and recommends that people stay outside of structures when they are being treated with chlorpyrifos and that a reentry interval be established before workers are allowed back inside. No residues were found on surfaces at this site 2 weeks after the application. This finding of poisoning by inhalation is contradicted by Dow Agrosciences scientist McCollister (1991) who stated that "acutely toxic levels of vapors cannot be attained at room temperature." However, no studies of human subjects exposed over a period of days have been located that would support this conclusion.

Zweiner and Ginsburg (1988) reported on 37 children seen in one hospital in Texas, ranging in age from 1 month to 11 years, with moderate or severe organophosphate poisoning. Ingestion of stored liquid was involved in 76% of cases and playing on carpet or floor after application was involved in 14% of cases. The initial diagnosis was not recognized as OP poisoning in 16 of the 20 children transferred for care. The most commonly reported symptoms included miosis (73%), excessive salivation (70%), muscle weakness (68%), respiratory distress (59%), lethargy (54%), nausea/vomiting (32%), seizures (22%) and coma (22%). Twelve (38%) of the children required mechanical ventilation to maintain respiration. Six of the total 37 cases were reportedly due to chlorpyrifos, more than any other organophosphate. Three of six chlorpyrifos cases were life threatening due to coma or respiratory arrest (Ginsburg, personal communication). The authors concluded that bradycardia and muscle twitching were less likely in childhood poisonings than in adults, but that seizures were more common in children. They noted that all children who had seizures also had respiratory insufficiency and that therefore hypoxia might be the underlying cause of the seizures.

Dow Agrosciences (1994) states that "In 20 years of manufacturing with regular monitoring of the workers' health status, we have never observed significant depression in red blood cell cholinesterase or symptoms of cholinesterase inhibition." This statement did not include information about the number of workers monitored or what signs or symptoms where checked. It suggests a safety record that contrast significantly with the experience of PCOs and consumers exposed to chlorpyrifos. HED is greatly concerned that users of chlorpyrifos may take this statement to mean they do not need to be careful about following safety instructions with chlorpyrifos and may falsely assume that any symptoms reported from exposure are coincidental rather than caused by the exposure. Such a casual approach could lead to disregarding common-sense safety precautions and legal requirements for safe use.

Chronic Effects

Summary

HED concludes that chlorpyrifos may be a significant cause of chronic neurobehavioral effects. Further study is needed to determine the prevalence, severity, and persistence of these effects, as well as the occurrence of self-reported multiple chemical sensitivity. The possibility that chlorpyrifos may also be a cause of peripheral neuropathy at sub-lethal doses has not been substantiated by the information collected for this review.

Case reports

Dr. Sheldon Wagner has served as a medical consultant for cases of illness potentially related to pesticides since the late 1980s. The Office of Pesticide Programs at EPA has provided funding for this consultation. In the first 20 months, Dr. Wagner consulted on over 300 referrals. The second most frequently raised concern, after chlordane, was chlorpyrifos which was responsible for 34 inquiries. Dr. Wagner noted "The most difficult problem has been encountered with chlorpyrifos. There have been 34 inquiries about this insecticide. The clinical problems most commonly raised have been complaints of long-term illness following acute exposure and/or intoxication (Wagner 1990)."

With the ban on chlordane in 1988, chlorpyrifos has become the number one source of referrals to Dr. Wagner. More recent reports specify the types of problems that are most common: An individual whose home was treated developed symptoms consistent with organophosphate poisoning. Dr. Wagner noted that the manner in which the PCO applied the product may have contributed to the problem: "It is my judgment that the label for Empire-20 is not clear as to whether this compound can be used in food dispensing areas such as the kitchen - as it was in this particular case. Furthermore, the label is also incorrect stating that any area in which the product has been applied may be treated simply by water (Wagner 1993)".

Another case reported in a school illustrates the potential for major costs associated with misapplication of Dursban: "This is another episode of acute illness developing in children as the result of pesticide treatment to a school in which the formulation was applied while children and teachers were in the building. Additionally, as is not unusual, the heat duct system became contaminated and illness became more severe when the heating system was turned on. This problem is similar to many other cases . . . many times the recommendation must simply be to put in an entirely new heat duct system (Wagner 1993)".

The following typical case of misuse was reported in 1994:
"Her home was treated by 'crack and crevice' in an excessive manner whereby Dursban (chlorpyrifos) was applied and freely flowed down the walls and also got onto furniture. It also was applied in an eating area. She developed complaints of dyspnea and diarrhea. she eventually was hospitalized with a diagnosis of organophosphate intoxication (Wagner 1994a)". Summarizing the chlorpyrifos problem in 1994, Dr. Wagner concluded: "The most frequent organophosphate concern continues to be from chlorpyrifos use within homes, not from agricultural practices (Wagner 1994b)".

Though rarely reported, the following case suggests chlorpyrifos potential to bring on asthma: "This was a child with no history of allergic or atopic problems. His room was treated with Dursban and he immediately developed an asthmatic syndrome which has been persistent. Documentation of an acute Reactive Airway Dysfunction Syndrome is excellent and correlates extremely well with the temporal relationship to the Dursban formulation (Wagner 1995)".

In a case reported directly to EPA by a physician, a worker was exposed to Dursban granules while mowing the lawn all day long without wearing a shirt. The high humidity combined with perspiration led to significant dermal exposure and symptoms consistent with organophosphate intoxication. One year later the worker still complained of persistent headaches, extreme muscle weakness, and problems with memory, concentration, confusion, irritability, and depression. **Labels for chlorpyrifos products applied to lawns need to be modified to warn people who may come into substantial contact with chlorpyifos before it dissipates.**

Cases reported to NPTN with possible chemical sensitivity

From 1984 through 1990 the National Pesticide Telecommunications Network (NPTN) received 1,022 calls complaining of unusual chemical sensitivity to pesticides. Many, perhaps the overwhelming majority of these calls, involved multiple chemical sensitivity type problems. Chlorpyrifos was the leading pesticide listed for chemical sensitivity, accounting for 158 calls during the 7 year period, or 15% of the total. Data from the 1990 survey of home and garden pesticide use permits a comparison based on the number of containers in U.S. homes (Whitmore et al. 1992) . The total number of pesticide containers was 247,650,000 and the total for chlorpyrifos was 16,652,000. The ratio of calls per million containers in U.S. homes was 9.5 for chlorpyrifos (158/16,652,000) and 4.1 for all pesticides (1,022/247,650,000). Although these ratios do not take into account the number of PCO applications in the home, it does appear that the chemical sensitivity problem associated with chlorpyrifos is not due to its widespread use.

Literature on chronic effects

Kaplan et al. (1993) reported that 5 of their 8 subjects with peripheral neuropathy experienced problems with memory and confusion suggesting central nervous system dysfunction. Other reviewers have questioned the diagnosis of peripheral neuropathy in these cases. Of the five cases with chronic neurobehavioral effects, four reported that they recovered after a period of months or years.

Rosenthal and Cameron (1991) reported that a 64 year old male had a termite application with chlorpyrifos and experienced severe abdominal pain, nausea, headache, difficulty breathing, fatigue, irritation of the eyes, nose and throat, anxiety, and irritability. Many of these symptoms reportedly continued for 2 years whenever he was present in the home. He also reported developing a sensitivity to new furniture and carpet odors.

Rouche (1988) reported on a 57 year old physician who was exposed to Dursban and Ficam (bendiocarb) when airing out a cabin that was treated monthly with these pesticides. Her initial symptoms included nausea, abdominal cramps, diarrhea, salivating, sweating, metallic taste in the mouth, tightness in chest, palpitations blurred vision, muscle weakness, twitching in legs, and tingling on bottom of feet. Persistent symptoms included leg weakness, decreased strength, muscle twitching, and reduced sensory response in the legs. She was diagnosed with peripheral neuropathy.

Steenland et al. (1994) performed a case-control study on 128 workers poisoned by organophosphates. Ten of these subjects had primary exposure to chlorpyrifos at the time of poisoning and an additional seven cases had poisoning from chlorpyrifos and some other organophosphate insecticide. Among those with primary poisoning from chlorpyrifos, they had significantly worse peroneal nerve conduction velocity and ulnar sensory amplitude. Those with any exposure involving chlorpyrifos reported more tension on mood scales and performed worse on tests of finger vibrotactile sensitivity.

Thrasher et al. (1993) reported on 12 chlorpyrifos victims 1-4.5 years after exposure. Their chief chronic complaints included fatigue, headaches, dizziness, loss of memory, joint and muscle pain, gastrointestinal disturbances, and respiratory symptoms. Eleven of the 12 cases involved application by a Pest Control Operator. A number of immunologic differences were reported in this population, but unfortunately these results have not been duplicated by other labs and recent literature has raised questions about the significance of the lab techniques employed.

The reports cited above provide evidence of neurobehavioral damage consistent with that reported for other organophosphate insecticides. The association is fairly specific and has been observed in a variety of different populations. Such effects are biologically plausible based on animal studies showing direct effects on the brain. Taking these case reports and studies together, it is reasonable to conclude that some subset of poisoned subjects probably experience persistent neurobehavioral effects as a result of their exposure to chlorpyrifos. The possible role of the odoriferous carrier which has been hypothesized to lead to a conditioned response should also be investigated. Currently, Poison Control Centers report 1100 symptomatic cases of exposure to chlorpyrifos annually. A prospective follow-up of these cases is needed to determine the prevalence, persistence, and severity of any chronic effects.

Reports of Birth Defects

There have been reports in the literature and reports sent to EPA suggesting an association between chlorpyrifos exposure and birth defects. These cases have been reviewed for EPA by the Division of Birth Defects and Developmental Disabilities at the National Center for Environmental Health, one of the Centers for Disease Control. Based on their review and other information, HED concludes the available evidence does not support a finding of teratogenicity based on human epidemiology studies and case reports.

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