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OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

Subject: Toxicology Review for the Reregistration Eligibility  
Document on Chlorpyrifos

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## CHLORPYRIFOS

### Acute Toxicity

Acute toxicity values and categories for chlorpyrifos are summarized in the following table.

TEST	RESULTS	CATEGORY
Oral LD <sub>50</sub> - rat	163 mg/kg m; 137 mg/kg F	II
Dermal LD <sub>50</sub> - rat	202 mg/kg	II
Eye Irritation - rabbit	slight irritation	III
Dermal Irritation - rabbit	slight irritation	III
Dermal Sensitization - guinea pig	non-sensitizing	

The oral LD<sub>50</sub> values for technical chlorpyrifos were 163 mg/kg in male rats and 137 mg/kg in female rats (toxicity category II) (Dow, 1963). The oral LD<sub>50</sub> values were lower in chicks at 32 mg/kg, toxicity category I, and in mice at 62.5 mg/kg, toxicity category II. The oral LD<sub>50</sub> in guinea pigs was 504 mg/kg, toxicity category III, and in rabbits was 1000 - 2000 mg/kg, toxicity category III (Dow, 1963). The dermal LD<sub>50</sub> in rats was 202 mg/kg, which is category II (Dow, 1963).

Application of chlorpyrifos to the rabbit eye resulted in slight irritation (category III) (Dow, 1963). A primary dermal irritation study indicated that application of chlorpyrifos produced slight hyperemia and slight burns on the skin, which healed by 21 days (category II) (Dow, 1963). No dermal sensitization occurred with chlorpyrifos in guinea pigs (Henck and Lockwood, 1978). Chlorpyrifos was not neurotoxic when given to hens at 50 mg/kg (the LD<sub>50</sub>) and at 100 mg/kg (Rowe et al., 1978). Another study found no neurotoxicity in hens at 110 mg/kg of chlorpyrifos (Roberts et al., 1987).

## Subchronic Toxicity

In one ninety-day study, chlorpyrifos was given to Sprague Dawley rats in dietary doses of 0, 0.5, 10, or 200 ppm. The systemic NOEL was 10 ppm (0.5 mg/kg/day) and the LOEL was 200 ppm (10.0 mg/kg/day). The LOEL was based on reduced body weights and slight decreases in the packed red cell volume, red cells, and hemoglobin, which were suggestive of slight anemia. A NOEL for cholinesterase inhibition was not obtained due to reductions in plasma enzyme in male rats at 0.5 ppm (0.025 mg/kg/day, lowest dose tested) (this study fills guideline 82-1; Crown et al., 1985).

In another rat study, Fischer 344 rats were given 0, 0.1, 1.0, 5.0, or 15 mg/kg/day chlorpyrifos in the diet. The systemic NOEL was 0.1 mg/kg/day. The LOEL in this study was 1 mg/kg/day, based upon increased organ weights (brain and heart), adrenal gland vacuolation, and reduced body weight gain at this and higher dose levels. The NOEL for cholinesterase inhibition was also 0.1 mg/kg; at 1 mg/kg (LOEL) decreases in plasma and red blood cell cholinesterase activities were noted (this study fills guideline 82-1; Szabo et al., 1988).

In the subchronic study with beagle dogs, the oral doses were 0, 0.01, 0.22, or 5.0 mg/kg/day. The NOEL was 0.01 mg/kg/day. The LOEL was 0.22 mg/kg/day, due to inhibition of plasma, red blood cell, and brain cholinesterase (this study fills guideline 82-1; Barker, 1989).

No effects were observed in a 21-day dermal study with Fischer 344 rats, which used chlorpyrifos in doses of 0, 0.1, 0.5, 1, and 5 mg/kg/day (this study fills guideline 82-2; Calhoun and Johnson, 1988). However, in a four-day dermal Fischer 344 rat study, (doses of 0, 1, 10, 100, or 500 mg/kg) reductions in plasma and red cell cholinesterase activities were seen at doses of 10 to 500 mg/kg/day. The NOEL was 1 mg/kg/day (Calhoun and Johnson, 1988).

Two subchronic inhalation studies of chlorpyrifos (nose-only exposure) were performed in Fischer 344 rats. The doses in the first were 0, 5.2, 10.3, or 20.6 ppb. The systemic toxicity and cholinesterase enzyme NOELs exceeded the highest dose tested of 20.6 ppb, or 287 ug/m<sup>3</sup> (Corley et al., 1986a and 1986b). In the second study the doses were 0, 5, 10, or 20 ppb. These systemic toxicity and cholinesterase enzyme NOELs also exceeded 20 ppb (highest dose tested) and a LOEL was not established (these studies fill guideline 82-4; Newton, 1988).

#### Chronic Toxicity and Carcinogenicity

The doses for beagle dogs in a two-year oral study were 0, 0.01, 0.03, 0.1, 1.0, or 3 mg/kg/day. The systemic LOEL was 3 mg/kg/day (highest dose tested) based upon an increase in liver weight. The NOEL's for inhibition of plasma, red blood cell, and brain cholinesterase activities were 0.01, 0.1, and 1.0 mg/kg/day, respectively (this study fills guideline 83-1; McCollister et al., 1971; Kociba, 1985).

Two carcinogenicity studies were performed with chlorpyrifos in rats. In one 2-year Fischer 344 rat feeding study, the doses were 0, 0.2, 5.0, or 100 ppm. The systemic toxicity NOEL was 5 ppm (0.33 mg/kg/day in males and 0.365 mg/kg/day in females). The LOEL was the highest dose tested (100 ppm or 6.99 mg/kg/day in males and 7.78 mg/kg/day in females) based upon reduced body weight in males and females and cataracts plus diffuse retinal atrophy in females. The NOEL for plasma cholinesterase inhibition was 0.2 ppm (0.0132 mg/kg/day, lowest dose tested) in males, where the LOEL was 5 ppm. The NOEL was less than 0.2 ppm in females since the LOEL was 0.2 ppm (0.0146 mg/kg/day), based on lower red blood cell cholinesterase. No compound related tumors were observed. (this study fills guidelines 83-1 and -2; Crown, 1990).

In another two-year feeding study, Fischer 344 rats were given 0, 0.05, 0.1, 1.0 or 10 mg/kg/day. The systemic NOEL was 1 mg/kg/day. The LOEL was 10 mg/kg/day, based on reduced weight gain; decreases in red cells, hemoglobin, cholesterol, protein and globulin; increases in platelets, and in urine specific gravity. There were also increases in adrenal gland weight, which microscopically showed fatty vacuolation of the zona fasciculata. The cholinesterase NOEL was 0.1 mg/kg/day and the LOEL was 1 mg/kg/day, due to decreased activities of plasma and brain enzyme activities. No compound related tumors were observed (this study fills guidelines 83-1 and -2; Young and Grandjean, 1988).

No carcinogenic or other toxic effects were observed in a two-year feeding study in CD-1 mice that tested dietary levels of 0, 0.5, 5, or 15 ppm (2.25 mg/kg/day) (this study fills guideline 83-2; Warner et al.; 1980)

Another oncogenicity study fed 0, 5.0, 50, or 250 ppm to CD-1 mice for 78 weeks. The systemic NOEL was 50 ppm (males 8.84 mg/kg/day, females 9.79 mg/kg/day). The LOEL was 250 ppm (males 45.2, females 48.1 mg/kg/day) based on decreased body weight in males and increased incidence of keratitis and hepatocytic fatty vacuolation. There were also decreased feed and water consumption, increased incidences of ocular opacity, and hair loss at this level. Plasma cholinesterase activities were reduced at all treatment levels; brain cholinesterase activities were decreased in the high-dose animals. Treatment-related tumors were not found (this study fills guideline 83-2; Gur, 1992).

#### Developmental Toxicity

Developmental toxicity studies on chlorpyrifos were conducted in rats, mice, and rabbits. In one CD rat study, gavage doses of 0, 0.5, 2.5 and 15 mg/kg/day were given on gestation days 6-15. The maternal systemic NOEL

was 2.5 mg/kg/day based upon reduced food consumption and body weight at 15 mg/kg/day. The developmental toxicity NOEL was also 2.5 mg/kg/day based on an increase in post-implantation loss at 15 mg/kg/day. Plasma cholinesterase activity was reduced in the dams at 0.5 mg/kg/day (this study fills guideline 83-3; Rubin et al., 1987a).

Chlorpyrifos was given by gavage to Fischer 344 rats at doses of 0, 0.1, 3, and 15 mg/kg/day on gestation days 6-15. Plasma and RBC cholinesterase inhibition occurred in the dams at 3 mg/kg/day (LOEL). No developmental toxicity occurred at any dose level (this study fills guideline 83-3; Ouellette et al., 1983).

Administration of chlorpyrifos to CF-1 mice at gavage doses of 0, 0.1, 1, 10, and 25 mg/kg/day on gestation days 6-15 resulted in a maternal systemic NOEL of 10 mg/kg/day. There were reductions in body weight and in food and water consumption plus increased mortality at the 25 mg/kg/day level. The developmental toxicity NOEL was also 10 mg/kg/day. This was due to minor skull variants, delayed ossification of skull bone and sternbrae, and reduced fetal body measurements at the highest dose level. Plasma and red blood cell cholinesterase activities were inhibited at 1 to 25 mg/kg/day in dams, for which the NOEL was 0.1 mg/kg/day. The same inhibitions were found at 10 and 25 mg/kg/day in fetuses, where the NOEL was 1 mg/kg/day (this study fills guideline 83-3; Deacon et al., 1979).

In New Zealand rabbits, gavage doses of 0, 1, 9, 81, and 140 mg/kg/day of chlorpyrifos on gestation days 7-19 resulted in a maternal systemic toxicity NOEL of 81 mg/kg/day. The LOEL was based on reduced food consumption and body weight gain, and apparent post-implantation loss at 140 mg/kg/day. Plasma cholinesterase activity was reduced in the dams at all dose levels, thus a NOEL for this factor was not established. The developmental toxicity NOEL was also 81 mg/kg/day. This was due to slight

decreases in fetal weights and crown-rump lengths, and an increased incidence of unossified xiphisternum or unossified 5th sternebra at 140 mg/kg/day (this study fills guideline 83-3; Rubin et al., 1987b).

### Reproduction

A two-generation dietary study in Sprague Dawley rats gave doses of 0, 0.1, 1.0, or 5.0 mg/kg/day. The parenteral systemic toxicity NOEL was 0.1 mg/kg/day (lowest dose tested). The LOEL was 1 mg/kg/day based upon cholinesterase inhibition in brain, plasma, and red cells, and histological lesions of the adrenal gland (vacuolation of cells of the zona fasciculata). The developmental NOEL was 1 mg/kg/day and the LOEL was 5 mg/kg/day (highest dose tested) as a result of reduced pup body weight and increased pup mortality (this study fills guideline 83-4; Breslin, 1991).

In a three-generation reproduction study, Sprague Dawley rats were given 0, 0.03, 0.1, 0.3, or 1.0 mg/kg/day in the diet. The maternal systemic NOEL was 0.1 mg/kg/day (lowest dose tested). The LOEL was 0.3 mg/kg/day due to reductions in plasma and red blood cell cholinesterase activities. No reproductive effects were observed at doses up to 1 mg/kg/day, the highest dose tested (This study fills guideline 83-4; Thompson, 1971).

### Mutagenicity

Chlorpyrifos did not produce gene mutation in several Ames reversion assays (Bruce and Zempel, 1986; Loveday et al, 1987) or in CHO/HGPRT assays in vitro (Mandralla, 1985; Tu, 1987). Also, it did not induce chromosome aberrations in vitro (Loveday, 1987) and it was not clastogenic in the mouse micronucleus test in vivo (Bhaskar et al., 1985). In other tests, chlorpyrifos did not induce unscheduled DNA synthesis in isolated rat



hepatocytes (Mandralla and Dryzga, 1986). A slight increase in recombination frequency in the Saccharomyces mitotic recombination assay (Simmon et al., 1977) and direct damage to DNA in a DNA repair assay using B. subtilis H17/m45 and E. coli pol A+/pol A- (Simmon et al., 1977) were noted. (These studies fill guidelines 84.)

#### Metabolism

Oral doses of 0.5 and 25 mg/kg of <sup>14</sup>C-chlorpyrifos were given to Fischer 344 rats in a metabolism and tissue distribution study. During 72 hours, more than 84% of the radioactivity was recovered in the urine, about 5% was found in the feces and less than 0.2% was in the tissues and carcass. The metabolism of chlorpyrifos was extensive, and no unchanged parent compound was found in the urine. The major urinary metabolites were 3,5,6-trichloro-2-pyridine (TCP), and glucuronide and sulfate conjugates of TCP (this study fills guideline 85-1; Nolan et al., 1987).

#### Special Studies

Chlorpyrifos was administered to sixteen male human volunteers. There were four volunteers in each group. They were given, by capsule, doses of 0, 0.014 or 0.03 mg/kg/day for 20 days, or a dose of 0.1 mg/kg/day for 9 days. The LOEL for plasma cholinesterase inhibition was 0.1 mg/kg/day and NOEL was 0.03 mg/kg/day (Coulston and Griffith, 1972).

#### Reference Dose (RfD) for Chronic Oral Exposure

The RfD for chlorpyrifos was determined to be 0.003 mg/kg per day. This was based on results of a human feeding study showing a NOEL for plasma cholinesterase inhibition of 0.03 mg/kg/day. An uncertainty factor of 10 was used.

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