Effects of Repeated Oral Postnatal Exposure to Chlorpyrifos on Open-Field Behavior in Juvenile Rats

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Organophosphorus (OP) insecticides have the potential to cause behavioral effects in children. This study was designed to determine if repeated oral exposure of preweanling rats to chlorpyrifos would produce behavioral changes at both pre- and postweanling ages. Treatment occurred every second day beginning on postnatal day (PND) 1, and continued through PND 21. The rats received one of the following regimens: a low-dosage (3 mg/kg) from PND 1–21; a medium dosage (mg/kg from PND 1–5, and then 6 mg/kg from PND 7–21; or a high-dosage schedule of 3 mg/kg on PND 1–5, then 6 mg/kg from PND 7–13, and 12 mg/kg from PND 15–21. There were no differences in body weights among the control-, low-, and medium-dosage groups but the high-dosage group had significantly lower body weights on PND 13–21. An open field was used to measure locomotor activity on PND 10, 12, 14, 16, 18, 20, 25, and 30. There were no differences in locomotor activity levels or treatment effects between males and females. On PND 10, 12, 14, 16, 18, and 20 there was no effect on locomotor activity with any dosage. On days 25 and 30, locomotor activity was significantly decreased with the medium- and high-dosage groups. Brain cholinesterase (ChE) inhibition was about 25–38% on PND 25 and 14–34% on PND 30. On PND 25 but not 30, lung and diaphragm ChE and serum butyrylcholinesterase (BChE), with the high-dosage animals, and heart ChE with the medium- and high-dosage groups were significantly inhibited. There was no significant inhibition of skeletal muscle ChE or serum acetylcholinesterase (AChE) on PND 25 and 30. These data suggest that early postnatal chlorpyrifos exposures will depress locomotor activity in juvenile rats, with the effects most pronounced after brain ChE activity has substantially recovered.

Key Words: acetylcholinesterase (AChE); neural developmental aberrations; chlorpyrifos; development; behavior; motor activity.

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Organophosphorus (OP) insecticides exert their toxicity through the inhibition of acetylcholinesterase (AChE). The inhibition of AChE leads to the accumulation of the neurotransmitter acetylcholine, causing hyperactivity in the central nervous system (CNS) and in neuromuscular junctions. In the CNS, acetylcholine plays an important role in the development of the pathways necessary for normal function, including cognitive function (Berger-Sweeney, 1998; Hohmann and Berger-Sweeney, 1998; Lauder and Schamba, 1999). Since these pathways are still developing in juvenile animals, any compound that alters the signals for development may disrupt the establishment of appropriate, functional pathways necessary for normal physiology and behavior. Thus, it is possible that exposure of juveniles to OP insecticides, which would elevate the levels of acetylcholine during development of the central nervous system, could result in developmental aberrations of neural pathways responsible for cognition as well as overall motor activity levels.

Chlorpyrifos, O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)phosphorothioate, is one of the most widely used OP insecticides. It is moderately toxic, with a rat oral LD₅₀ of 135–163 mg/kg (Worthing and Walker, 1987), and is categorized as Class II toxicity by the U.S. Environmental Protection Agency. It has numerous agricultural applications and was, until its recent restrictions, one of the most popular insecticides for termite control and general residential indoor/outdoor insect control. Because of its widespread use, it was a compound to which many Americans were frequently exposed. The concern with insecticide exposure in juveniles (Fenske et al., 1990) and the frequent widespread application of chlorpyrifos have sparked interest in its developmental toxicity.

Many recent investigations have focused on the determination of the mechanisms behind the age-related toxicity differences, since juveniles are more susceptible (Atterberry et al., 1997; Chakrabarti et al., 1993; Mortensen et al., 1996; Pope and Chakrabarti, 1992; Pope et al., 1991), and on the neurochemical alterations resulting from repeated exposure during development (Campbell et al., 1997; Song et al., 1997; Whitney et al., 1995). However, there is a limited amount of data concerning the effects of juvenile chlorpyrifos exposure on behavior. Acute gestational exposure has been shown to produce a transient effect in righting reflex in pups on PND 1 and 3 (Chanda et al., 1995). Repeated gestational exposure produced deficits in righting reflex and cliff avoidance on PND 1 and 3 (Chanda and Pope, 1996) and rotarod performance on PND 16 (Muto et al., 1992). Acute exposure of weanling rats...
to a high dose (120 mg/kg) of chlorpyrifos on PND 21 produced selective learning deficits (Stanton et al., 1994). However, acute exposure to lower levels (0.3 mg/kg) of chlorpyrifos to pups on PND 3, 10, and 12 did not yield any deficits (Muto et al., 1992). Repeated subcutaneous injections of chlorpyrifos on PND 7, 11, 15, and 19 did not yield any locomotor activity deficits (Chakraborti et al., 1993). Acute oral exposure to pups on PND 17 produced effects on open-field arousal (Moser et al., 1998) and open-field activity (Moser and Padilla, 1998) as well as other functional observational battery changes. More recently, daily subcutaneous injection of 1 mg/kg chlorpyrifos on PND 1–4 resulted in decreased reflex righting (PND 3–4) and negative geotaxis (PND 5–8) performance in female rats but not males (Dam et al., 2000). Conversely, locomotor activity and rearing (PND 21 and 30) were decreased in males but not females. These results suggest that there are sex differences in the effects of chlorpyrifos with respect to behavior.

While subcutaneous injection could mimic dermal exposure, it does not appropriately consider the disposition and metabolism of the insecticide when penetration through the skin is a determining factor. Confounding issues such as the maternal grooming of young rats by the dam precludes direct dermal application as a route of exposure. Thus, the oral route was selected for the present study, which would be more relevant to environmental-exposure situations. There is currently no applicable literature on the behavioral impact of repeated oral developmental exposures of neonates to chlorpyrifos.

This project was designed to determine if repeated oral exposure of rats to chlorpyrifos during preweaning ages would produce any changes in open-field motor activity at both the pre- and postweaning ages. In addition, the levels of ChE inhibition in the central and peripheral nervous system tissues, including serum AChE and BChE, which are used as common biomarkers of OP exposure, were determined to correlate with the behavioral results. The exposure protocol and dosage levels were made similar to those previously used (Tang et al., 1999), in order to be able to compare the previously reported muscarinic receptor data with behavioral data.

**MATERIALS AND METHODS**

**Chemicals.** Analytical grade chlorpyrifos was the generous gift of Dow Agrosciences (Indianapolis, IN). Other biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

**Animal treatments.** Adult male and female Sprague-Dawley [Crl:CD(SD)BR] rats from Charles River were used as breeders. All animals were kept in a temperature-controlled (22 ± 2°C) room with a 12:12h alternating light/dark cycle in an AAALAC-accredited facility. Animals were allowed free access to food (standard laboratory rodent feed) and water. All procedures were approved previously by the Mississippi State University Institutional Animal Care and Use Committee. Following parturition, litters were celled to 8 pups (4 females and 4 males when possible) and maintained at 8 during behavioral testing in the preweaning ages to ensure similar nutritional availability in all litters. An entire litter was randomly assigned to one of the 4 treatment groups. The experiment was repeated 3 times, with 8 litters (2 litters per treatment) present during the first and second runs and 4 litters (1 litter per treatment) during the third run. Each pup was individually treated, and from each litter, two males and two females were used for behavioral testing. Thus, for all experiments, there were a total of 10 males and 10 females used for behavioral testing for each treatment group.

Chlorpyrifos dissolved in corn oil was administered to rat pups every other day by oral gavage at a volume of 1 ml/kg beginning on PND 1 (the day of birth was counted as PND 0) and continuing through PND 21. Pups were treated between the hours of 1300 and 1600 (in the light phase). The treatment groups were: (1) control, which was administered the corn oil vehicle only; (2) low-dosage, administered a constant dosage of chlorpyrifos (3 mg/kg) every other day from PND 1 through 21; (3) medium-dosage, administered 3 mg/kg every other day from PND 1 through 5, and then 6 mg/kg every other day from PND 7 through PND 21; and (4) high-dosage, administered 3 mg/kg every other day from PND 1 through PND 5, 6 mg/kg every other day from PND 7 through PND 13, and then 12 mg/kg every other day from PND 15 through PND 21.

**Behavioral testing.** The open field apparatus was constructed of plexiglass (45.72 cm × 45.72 cm with 22.86 cm walls) and was divided by transversing lines into 7.62 cm squares. The box was warmed to nest temperature (33°C) by heating pads placed under the box. Lighting during testing was provided by two 15W fluorescent lights. A video camera was mounted above the box to record activity. The animals were tested during the light phase (0800–1000) on PND 10, 12, 14, 16, 18, 20, 25, and 30. The order of testing was altered, such that each of the 4 treatment groups entered the maze in the same order only twice during the experiment. For example, controls were tested first only on days 10 and 18, low-dose animals on days 12 and 20, medium on days 14 and 25, and high on days 16 and 30.

Testing was initiated by placing the rat in the center of the box. Each rat was placed in the same square facing the same direction for each testing period. Activity was monitored for 3 min on PND 10–12 and for 6 min on PND 14–30. Two individuals, who were blind to treatment, observed the video independently and each recorded the number of line crosses. A line cross was counted when both front feet of an animal entered an adjacent square. The 2 values obtained for each pup at each age were averaged to a single value. The maze was cleaned between each test.

**Tissue sampling.** Additional non-behavioral litters were assigned to treatment groups. Each litter received a single treatment. The rats were used to obtain tissue for biochemical analysis at one time prior to behavioral testing and several times corresponding to the time of behavioral testing during the light phase (0800–1000). At these selected time points (PND 6, 10, 16, 20, 25, and 30), a single male and female were removed from each litter and were euthanized by decapitation. Blood, brain, lungs, whole heart, skeletal muscle (quadriceps), and diaphragm were immediately removed. The brains were dissected into the forebrain and hindbrain (medulla-pons and cerebellum). The blood was centrifuged for 6 min at 17,000 g to obtain serum. All tissues and serum samples were frozen at −70°C until ChE determination.

**Esterase assays.** All tissues were homogenized in ice-cold 0.05 M Tris–HCl buffer (pH 7.4). Brain regions were homogenized using a motorized Wheaton pestle and glass mortar. Peripheral tissues were homogenized using a Polytron at a setting of 6 for 1 min. The final tissue concentrations were 1 mg/ml for forebrain and hindbrain, 1.875 mg/ml for lung, 0.8 mg/ml for heart, 1.5 mg/ml for skeletal muscle, 1.25 mg/ml for diaphragm, and 10 μl/ml for serum. Forebrain, hindbrain, lung, heart, skeletal muscle, and diaphragm ChE were measured spectrophotometrically using a modification (Chambers et al., 1988) of Ellman et al. (1961) using 1.0 mM acetylthiocholine as the substrate and 5.5'-dithio-bis(nitrobenzoic acid) as the chromogen. Eserine sulfate (10 μM), a ChE inhibitor, was used to correct for non-ChE hydrolysis. Serum AChE was determined similarly but all tubes were preincubated with 10 μM iso-OMPA, a specific BChE inhibitor. Serum BChE was measured similarly with 2.0 mM butyrylthiocholine as the substrate and 10 μM iso-OMPA to correct for non-BChE hydrolysis. Protein content of the tissues was quantified with the Folin phenol reagent using bovine serum albumin as a standard.
Specific activities were calculated as nmol product formed/min mg protein.

**Statistical analysis.** For biochemical and behavioral measures, data were analyzed using SAS on a personal computer by analysis of variance (General Linear Model) for a repeated-measures design with one between-litter factor and two within-litter factors. Treatment effects were tested using the mean square for litter-within treatment. Age, sex, sex × treatment, age × sex, age × treatment, and age × sex × treatment were tested by the mean square error. As expected for a developmental study, age effects were present and mean separation of control values at different ages were determined using the least-squares means method. However, there were no sex effects and no significant interactions were detected for sex × treatment, age × treatment, and age × sex × treatment. Data were then analyzed separately by age. Mean separation was determined using the least-square means method using the error term appropriate for the effect tested. Statistical significance is reported for the \( p \leq 0.01 \) level. For mortality, frequency data were analyzed by Fisher's test for uncorrelated proportions.

**RESULTS**

Overt signs of toxicity (tremors) were observed 2 h after pups were administered chlorpyrifos orally with the first high-dosage treatment on PND 15, but lesser signs were observed after the subsequent administrations of the high dosage. No overt signs were observed in the low- or medium-dosage groups. A small number of deaths occurred in all treatment groups (Table 1) at various points during the treatment period, with no significant differences between treatments. On days 13 through 21, body weights of pups in the high-dosage group were significantly lower than weights of controls (Fig. 1). However, by PND 25 and 30, activities in the high- and medium-dosage groups were significantly decreased in a dose-related manner in both males and females.

In control and treated animals, specific activities of forebrain, hindbrain, skeletal muscle, lung, heart, and diaphragm ChE, serum AChE, and serum BChE were not significantly different between male and female pups, thus data from both sexes for each treatment group were pooled for statistical analysis. However, there were tissue-specific developmental changes in the control-specific activities of ChE.

Forebrain (Fig. 3A) and hindbrain (Fig. 3B) control ChE-specific activities increased significantly through PND 20, then remained stable through PND 25. There was a significant increase in control activity of forebrain ChE on PND 30, but there was a significant decrease in control activity of hindbrain ChE on PND 30. Following treatment, forebrain and hindbrain ChE was significantly inhibited in a dose-related manner in all 3 treatment groups at all ages tested. With the exception of PND 6, ChE inhibition in either brain region did not exceed 50%.

Skeletal muscle ChE control-specific activity (Fig. 4A) decreased with age and did not appear to reach a stable level by PND 30. Following treatment, skeletal muscle ChE-specific activity was significantly inhibited on PND 6, 10, and 16 in the low-dosage group and on PND 10 and 16 in the medium-dosage group. Both low and medium groups were similar to

![FIG. 1. Change in body weight in rat pups during repeated oral exposure to 3 dosages of chlorpyrifos from PND 1 through 21, as described in Materials and Methods. Values are expressed as mean ± SE (n = 20); *significantly different from control (p ≤ 0.01).](image-url)
control levels by PND 20. In the high-dosage group, ChE was significantly inhibited on PND 16 and 20 but was similar to control levels by PND 25. Skeletal muscle ChE inhibition did not exceed 40% in any treatment group.

Lung ChE control-specific activity (Fig. 4B) decreased with age and reached a stable level by PND 20. Following treatment, lung ChE-specific activity was significantly inhibited on PND 6, 10, 16, and 20 in the low-dosage group and on PND 10, 16, and 20 in the medium-dosage group. Both low and medium groups were similar to control levels by PND 25. In the high-dosage group, ChE was significantly inhibited on PND 16, 20, and 25, but was similar to controls by PND 30. Lung ChE inhibition exceeded 50% on PND 16 and 20 in all treatment groups.

Diaphragm ChE control-specific activity (Fig. 5B) decreased with age and reached a stable level by PND 16. Following treatment, diaphragm ChE-specific activity was significantly inhibited on PND 6, 10, 16, and 20 in the low-dosage group but was similar to control levels by PND 20. In the medium-dosage group, ChE was significantly inhibited on PND 10, 16, and 20, but was similar to controls by PND 25. In the high-dosage group, ChE was significantly inhibited on PND 16, 20, and 25.

Heart ChE control-specific activity (Fig. 5A) did not change with age. Following treatment, heart ChE specific activity was significantly inhibited in the low dosage group on PND 6, 10, 16, and 20, but was similar to control levels by PND 25. ChE was significantly inhibited on PND 10, 16, 20, and 25 in the medium dosage group and on PND 16, 20, and 25 in the high dosage group. Both medium and high groups were similar to control levels by PND 30. Heart ChE inhibition exceeded 50% on PND 16 and 20 in all treatment groups.

Diaphragm ChE control-specific activity (Fig. 5B) decreased with age and reached a stable level by PND 16. Following treatment, diaphragm ChE-specific activity was significantly inhibited on PND 6, 10, and 16 in the low-dosage group but was similar to control levels by PND 20. In the medium-dosage group, ChE was significantly inhibited on PND 10, 16, and 20, but was similar to controls by PND 25. In the high-dosage group, ChE was significantly inhibited on PND 16, 20, and 25.

FIG. 2. Open field activity in female (A) and male (B) rat pups following repeated oral exposure to 3 dosages of chlorpyrifos from PND 1 through 21, as described in Materials and Methods. Values are expressed as mean total number of line crosses $\pm$ SE ($n = 10$); *significantly different from control ($p \leq 0.01$).

FIG. 3. Forebrain (A) and hindbrain (B) cholinesterase activity following repeated oral exposure to 3 dosages of chlorpyrifos from PND 1 through 21, as described in Materials and Methods. Values are expressed as mean $\pm$ SE ($n = 6–8$). Percent inhibition for each statistically significant value is presented in the oval overlaying the corresponding bar. Capital letters indicate significant differences among control values ($p \leq 0.01$). Within each age, bars not labeled with similar lower case letters are significantly different ($p \leq 0.01$).
but was similar to controls by PND 30. Diaphragm ChE was inhibited about 50% on PND 10, 16, and 20 in the high-dosage group.

Serum AChE control-specific activity (Fig. 6A) initially increased from PND 6 to PND 10 then decreased thereafter with age. Activity did not appear to reach a stable level by PND 30. Following treatment, serum AChE-specific activity was significantly inhibited on PND 6 and 10 in the low-dosage group but was not significantly different from control levels by PND 25. Serum AChE was significantly inhibited on PND 10, 16, and 20 in the medium-dosage group and on PND 10, 16, and 20 in the high-dosage group. Both low and medium groups were similar to control levels by PND 25. Specific activity was significantly inhibited on PND 16, 20, and 25 in the high-dosage group but was similar to controls by PND 30. The greatest levels of inhibition in this experiment were observed with serum BChE, with inhibition of up to 89% observed.

DISCUSSION

The age-dependent increase in forebrain and hindbrain ChE-specific activity was similar to that previously reported (Atter-
berry et al., 1997; Fiedler et al., 1987; Hrdina et al., 1975; Kristt, 1983). The age-dependent changes in peripheral tissue ChE have not previously been reported. Additionally, it appears that certain peripheral tissues may be more susceptible than others to the inhibitory effects on ChE occurring during chlorpyrifos exposure. For example, on PND 16 and 20, there was substantial inhibition (> 50%) of ChE in the lung and heart and of BChE in the serum. There was moderate inhibition (43–50%) of ChE in the diaphragm, but inhibition of skeletal muscle ChE and serum AChE was lower (< 40%).

This study was designed to investigate the motor-activity effect of repeated exposure of juvenile animals to chlorpyrifos. During most of the exposure period (PND 1–20), no significant disruption of motor activity was observed in the rat pups. The lack of behavioral deficits in the younger rats (PND 1–16) is understandable, considering the low activity of pups of these ages. It is logical to assume that the open-field behavioral test is not sensitive enough to be utilized for testing the behavioral effects of toxicants in very young preweanling animals. On PND 18 and 20, the activity of the pups should have been sufficient to detect any significant negative impact on motor activity resulting from the chlorpyrifos exposure. However, no significant decreases in open-field activity were observed with any treatment during these ages, even though exposure was ongoing. The only suppression of activity was observed on PND 25 and 30 in the medium- and high-dosage groups, which were 4 and 9 days following cessation of exposure, respectively. This suggests that repeated developmental exposure to chlorpyrifos can result in long-term deficits in locomotor activity, but further study is required to determine if locomotor deficits persist beyond PND 30.

One possible explanation for the deficits in motor activity is negative effects on the peripheral musculature, which would limit the mobility of the pups in the open field. However, there was no significant inhibition of skeletal muscle ChE present on PND 25 and 30, times when the deficits in activity were observed. Additionally, while significant inhibition of lung, heart, and diaphragm ChE activity and serum BChE activity was present on PND 25, there was no significant inhibition of enzyme activity in any peripheral tissue on PND 30. Thus, the behavioral deficits observed in the open field cannot be explained by decreased mobility resulting from chlorpyrifos-induced inhibition of peripheral ChE. However, necrosis of skeletal muscle fibers must be considered as a basis for the decreased mobility observed here. Such necrosis has been observed following acute exposure to the OP insecticide isofenphos and the insecticide metabolite paraoxon (Calore et al., 1999; Dettbarn, 1984). The severity of the necrosis is thought to be dependent on the level and duration of inhibition of ChE. However, no information is available on whether or not necrosis would occur following persistent inhibition of skeletal muscle ChE in developing animals.

Previous studies reported decreased open-field activity in rat pups following acute oral exposure to chlorpyrifos on PND 17 (Moser and Padilla, 1998). Similar behavioral changes were observed in male and female rats with effects occurring from 6.5 to 24 h post-exposure and recovery thereafter. The time when behavioral deficits were observed correlated very well with the time of maximal inhibition of peripheral and brain ChE. In contrast, the deficits observed in the current study, following repeated exposure to chlorpyrifos in younger ages of rats, do not correlate with inhibition of brain or peripheral ChE. In fact, brain ChE inhibition at the time when behavioral deficits were observed was lower than previous ages when no deficits were observed.

Sex differences in locomotor activity, such as those observed following a single subcutaneous administration of chlorpyrifos to rats on PND 1–4 (Dam et al., 2000), were not observed in the present study, which utilized an oral exposure
route. In addition, Dam et al. (2000) reported sex differences in AChE inhibition on PND 2, following exposure on PND 1 using the subcutaneous route. However, no sex differences in AChE inhibition were present in this study on PND 6. The present study did not determine AChE inhibition on PND 2. Future studies are needed to investigate possible sex differences in both inhibition of AChE at early ages and decreased behavioral performance with respect to route of administration. We are not aware of any theoretical reasons why sex differences in these parameters would be expected following exposure to an OP insecticide at these ages.

Significant decreases in muscarinic receptor numbers following chlorpyrifos exposure in juvenile rats have been reported (Moser and Padilla, 1998; Stanton et al., 1994), while others reported on marginal changes (Chakraborti et al., 1993). We have previously reported changes in the density of muscarinic receptors in the brains of rats exposed to chlorpyrifos using an identical exposure regimen as in the present study (Tang et al., 1999). In the medium- and the high-dosage groups, there was a significant decrease in the total brain muscarinic receptor density only on PND 22, using the lipophilic compound [3H]-quinuclidinyl benzilate as a ligand. Using the hydrophilic compound [3H]-N-methylscopolamine as a ligand, the density of muscarinic receptors present on the cell surface was decreased on PND 6 in the low-dosage group, on PND 14, 22, and 25 in the medium-dosage group, and on PND 22 and 25 in the high-dosage group. The decrease in muscarinic receptors following OP exposure is commonly associated with the development of behavioral tolerance (Hoskins and Ho, 1992). In fact, decreased muscarinic receptor numbers in the brain and behavioral tolerance have been demonstrated following acute exposure of adult rats to chlorpyrifos (Bushnell et al., 1993). In our previous study (Tang et al., 1999), the decreased muscarinic receptor numbers in the brain of chlorpyrifos-treated rats suggests that biochemical tolerance has occurred. However, the presence of behavioral deficits reported here, occurring at the same time as significant decreases in cell surface muscarinic receptor numbers (Tang et al. 1999) on PND 25, does not indicate that behavioral tolerance is associated with the ongoing biochemical tolerance. Following repeated exposure to chlorpyrifos in adults, it has been suggested that there is a functional cost of maintaining the biochemical tolerance and that this cost manifests itself as deficits in motor skills (Bushnell et al., 1994). The data presented here suggests that this may be true for young animals as well as adults.

It has been proposed that considerable brain ChE inhibition must be obtained (≥ 50%) before behavioral deficits will occur with chlorpyrifos (Moser et al., 1998; Moser and Padilla, 1998; Nostrandt et al., 1997). However, in the present study, behavioral deficits were obtained when brain ChE inhibition was at its maximum 38% and ChE activity was recovering. Likewise, these deficits occurred during times when the numbers of surface muscarinic receptors were reduced (PND 25) and were similar to control levels (PND 30) (Tang et al., 1999). The lack of correlation between biochemical changes and behavioral deficits suggests that the repeated exposure to chlorpyrifos has resulted in some other neurological disruption or physical (i.e., necrosis) that decreased the overall activity of the animal. With the recent reports concerning the chlorpyrifos-induced decrease in cell numbers and cell signaling in the brain of juvenile rats (Campbell et al., 1997; Song et al., 1997), it is possible that the decreased activity observed here may be the result of such effects on brain development. However, it is not known if these behavioral deficits are temporary and overall activity will recover or if they are persistent and are an indication of a permanent neurological dysfunction induced by the early chlorpyrifos exposure. Further investigation of the behavioral effects of repeated exposure to OP insecticides to juveniles is required.

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REFERENCES


