Developmental Chlorpyrifos and Methyl Parathion Exposure Alters Radial-Arm Maze Performance in Juvenile and Adult Rats

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Although the use of organophosphate (OP) insecticides has been restricted, sufficient exposure can occur to induce detrimental neurobehavioral effects. In this study, we measured physical and reflex development and spatial learning and memory in rats repeatedly exposed to incremental doses of chlorpyrifos (CPS) and methyl parathion (MPS) from postnatal day (PND) 1 to PND21. Other than decreased body weight in the higher dosage groups, no effects on physical or reflex development were observed. Significant hippocampal cholinesterase inhibition was induced in all treatment groups for up to 19 days following exposure. Beginning on PND36, working and reference memory was tested using a 12-arm radial maze, with subject animals trained and tested 4 days a week for 4 weeks. In males, working memory was decreased with the medium and high dosage of CPS but only the high dosage of MPS; while in females, no deficits were observed. For reference memory, errors were significantly increased in males exposed to the high dosage of CPS and all dosages of MPS. In females, enhanced performance was observed within the medium and high dosages of CPS but not with MPS. These data show that repeated developmental exposure to OP insecticides can induce sex-selective alterations and long-lasting changes in spatial learning and memory formation when measured using a radial arm maze and that MPS and CPS induce different neurobehavioral outcomes.

Key Words: chlorpyrifos; methyl parathion; behavior; developmental; pesticides.

In spite of the many stringent federal regulations restricting the use of chlorpyrifos (CPS) and methyl parathion (MPS) to agricultural applications, these organophosphate (OP) insecticides remain widely used. Accordingly, applicators, farm families, and the general public are still being exposed to appreciable quantities of CPS and MPS as indicated by the presence of their metabolites in body fluids (Adgate et al., 2001; Barr et al., 2004, 2005; NHANES, 2005). This potential exposure to CPS or MPS residues from agricultural use and other sources, such as food, suggests that they may pose a potential neurotoxicity risk, especially to brain development in children (Lu et al., 2006; Morgan et al., 2005).

Historically, exposure to OPs, such as CPS or MPS, targets the cholinergic system by binding to and inhibiting acetylcholinesterase (AChE). Inhibition of AChE leads to the accumulation of acetylcholine, the subsequent overstimulation of the cholinergic receptors, and finally the presentation of clinical symptoms including cognitive changes. However, previous studies have repeatedly reported that repeated exposure of neonatal rats to CPS at subthreshold dosages that do not elicit overt signs of toxicity can produce behavioral and cognitive deficits (Aldridge et al., 2005a; Levin et al., 2001). With the exception of Gupta et al. (1985), few studies investigating the effects of repeated developmental exposure to MPS on learning and memory are available.

It has been reported that multiple neurotransmitter systems are altered following exposure to OP insecticides. Developmental CPS exposure produces persistent deficiencies in cholinergic synaptic neurochemistry (Aldridge et al., 2005a; Dam et al., 1999; Guo-Ross et al., 2007; Liu et al., 1999; Richardson and Chambers, 2005; Slotkin et al., 2001; Tang et al., 1999) as well as persistent effects on serotonin and catecholamine neurochemistry (Aldridge et al., 2003, 2004, 2005a,b,c; Dam et al., 1999; Slotkin and Seidler, 2005, 2007; Slotkin et al., 2002). Developmental MPS exposure can also lead to deficiencies in cholinergic synaptic neurochemistry (Guo-Ross et al., 2007; Liu et al., 1999; Tang et al., 2003) and effects on serotonin and catecholamine neurochemistry (Mahaboob et al., 2001), but the persistence of the effects induced by MPS is not clear. Although it is apparent that repeated developmental exposure to OPs produces changes in both cholinergic and non-cholinergic pathways, the mechanism responsible for these changes is unclear.

It has been demonstrated that low-dose postnatal exposure of rats to different OPs, such as CPS, diazinon, and parathion, can have quite different neurotoxic effects and result in different patterns of memory impairment (Levin et al., 2001; Timofeeva et al., 2008a,b). It has been suggested that these effects are unconnected to their shared property as cholinesterase inhibitors. However, even though all OPs inhibit cholinesterase,
EARLY CPS AND MPS EXPOSURE ALTERS SPATIAL MEMORY IN RATS

Considerably different between compounds. Would differ following exposure to CPS and MPS, the ultimate radial maze. CPS or MPS on spatial learning and memory using the 12-arm designed to compare the effects of developmental exposure to their ability to induce neurocognitive deficits. This study was inhibition, aging, and recovery, it is of interest to compare cholinergic system, and the cholinergic system has been known cholinergic and non-cholinergic neural pathways including those that are essential to the integration of spatial learning, long-term potentiation, and memory.

Traditionally, OP insecticides exert their effects on the cholinergic system, and the cholinergic system has been known for some time to play a critical role in spatial learning and spatial memory (D’Hooge and De Deyn, 2001). Given that CPS and MPS differ in their patterns of cholinesterase inhibition, aging, and recovery, it is of interest to compare their ability to induce neurocognitive deficits. This study was designed to compare the effects of developmental exposure to CPS or MPS on spatial learning and memory using the 12-arm radial maze.

Since the patterns of stimulation in the nervous system would differ following exposure to CPS and MPS, the ultimate impact on working memory and reference memory could be considerably different between compounds.

MATERIALS AND METHODS

Chemicals. CPS (> 99% purity) was a generous gift from DowElanco Chemical Company (Indianapolis, IN). Analytical grade MPS (> 97% purity) was supplied by Dr Howard Chambers (Department of Entomology and Plant Pathology, Mississippi State University). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO).

Animals. Adult male and female Sprague-Dawley rats (CD IGS) from Charles River Laboratories (Wilmington, MA) were used as breeders. All animals were housed in a temperature-controlled room at 22°C ± 2°C with a 12:12 h light/dark cycle in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. LabDiet rodent chow (Richmond, IN) and tap water were available ad libitum during the breeding and growth period prior to behavioral testing in the radial maze. All procedures were previously approved by the Mississippi State University Institutional Animal Care and Use Committee. After a 14-day acclimation, male and females were bred at a ratio of 1:2 for 5 days and then separated. The size of the litter was adjusted as much as possible in order to obtain litters of the same size (12–14 pups) and even distribution of male and female pups within each litter. Day of birth was designated postnatal day (PND) 0. Following parturition, male and female rat pups within the same litter were randomly assigned to different treatment groups. Attempts were made to include all seven treatments for a single sex within a litter, but there were seven litters which did not contain all treatments for a single sex. In some cases, not all treatments were present in each litter, but there was always a representative control animal present for each sex in each litter. For this project, rats from 20 litters were used for behavioral testing. Not all rats within a litter were used for behavioral testing, but these litters were never removed prior weaning. Their physical and reflex data were not included in the statistical analysis in the project. The number of rats of each sex in each treatment group is indicated in Table 1.

Animal treatments. The insecticides were dissolved in corn oil at a volume of 0.5 ml/kg body weight and, using an incremental dosing regimen, were administered by oral gavage daily to Sprague-Dawley rats from PND1 through PND21 as described in Table 1. Control animals received corn oil daily. Oral gavage was performed using a 25-μl tuberculin syringe equipped with a 1-inch 24-gauge straight intubation needle (Popper and Sons, Inc., New Hyde Park, NY) to deliver the solution to the back of the throat. The dosing regimen was designed to avoid OP-induced mortality and overt signs of toxicity but to maintain moderate levels of whole-brain inhibition throughout the exposure period. The regimen is similar to previously published studies (Richardson and Chambers, 2005; Tang et al., 1999, 2003) although lower dosages of CPS and MPS were used here. It is unclear if these dosages are within the range of exposure levels in children. Most likely, CPS and MPS exposure in children will occur at levels lower than those used in this study in the exception of situations which involve high levels of contamination.

Hippocampal cholinesterase. For cholinesterase (ChE) determination, cohorts of male and female rats were treated and sacrificed on PNDs 20, 30, 40, 50, and 60. The hippocampus was removed, frozen in liquid nitrogen, and stored at −80°C until assay. For assay, hippocampal samples were thawed on ice and homogenized in 0.05M Tris-HCl buffer (pH 7.4 at 37°C). Cholinesterase activity was determined using a continuous assay with acetylthiocholine as the substrate and 5,5′-dithio-bis(nitrobenzoic acid) as the chromogen according to the method of Ellman et al. (1961). Eserine sulfate (10−5M) was used to correct for non-ChE-mediated substrate hydrolysis. Specific activity was calculated and expressed as nanomoles product produced per minute milligram protein. Protein concentration was measured using the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Physical and reflex development. Each day, beginning on PND1, all pups were observed for the following physical parameters: body weight, pinna detachment ( unfolding of the external ear), downy fur development, hair growth, incisor eruption, and eye opening. The following reflex tests (Smart and Dobbing, 1971) were assessed in all pups: surface righting, negative geotaxis, cliff avoidance, free fall righting, and acoustic startle. For surface righting, pups were directly placed on their back on a flat tabletop and released. The time was recorded until all four paws were placed on to the table surface. For negative geotaxis, pups were placed in a head-down position in the middle of a board covered with small wire mesh (5 mm) inclined at 45° from the surface plane. The amount of time to turn 180° to a head-up position was recorded. For cliff avoidance, pups were placed on the edge of a cliff with the forepaws and face over the edge. The amount of time for the pup to move away from the cliff by backing up or by turning sideways was recorded. For acoustic startle (a whole-body startle in response to a sharp clicking noise) and free fall righting (ability of a pup to correct its posture such that it lands on all four feet when released from a supine position 30 cm above a soft foam surface), the time of appearance was determined.

Radial-arm maze procedure. To test working and reference memory, a 12-arm radial maze interfaced with a computer for data collection was used (Columbus Instruments, Columbus, OH). The procedure adapted was...
**TABLE 1**
Dosages Administered in the Incremental Dosing Regimen and Number of Rats Undergoing Behavioral Testing in Each Treatment Group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>PND1–PND5</th>
<th>PND6–PND13</th>
<th>PND14–PND20</th>
<th>Behavioral Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Oil</td>
<td>Oil</td>
<td>Oil</td>
<td>13  14</td>
</tr>
<tr>
<td>MPS low (mg/kg)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>10  12</td>
</tr>
<tr>
<td>MPS medium (mg/kg)</td>
<td>1.0</td>
<td>2.0</td>
<td>4.0</td>
<td>10  12</td>
</tr>
<tr>
<td>MPS high (mg/kg)</td>
<td>1.5</td>
<td>3.0</td>
<td>6.0</td>
<td>11  12</td>
</tr>
<tr>
<td>MPS low (mg/kg)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>10  10</td>
</tr>
<tr>
<td>MPS medium (mg/kg)</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>10  10</td>
</tr>
<tr>
<td>MPS high (mg/kg)</td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
<td>9   10</td>
</tr>
</tbody>
</table>

a modification of Daniel et al. (1997), Levin et al. (2001), and Mizuno et al. (2000). The maze was made from black-painted metal sheeting and located in a quiet room with multiple extramaze visual cues strategically placed throughout the room. The maze consisted of a 34-cm circular platform (a central hub) with 12 arms (65 cm long × 9.5 cm wide with 25 cm walls) extending radially from the center. Each arm was partially enclosed by a dark sloping barrier (18 cm high at the central hub, descending to a height of 8 cm at the end) that extended 34 cm into the arm which prevented the rats from entering adjacent arms without reentering the central hub.

Following exposure, rats were tested in the maze from PND29 to PND60, a period that encompassed juvenile and adolescent stages of development. Beginning on PND29, rats were “habituated” for 4 days with a single session per day. "Habituation" involved placing each rat in the central hub of the maze with all 12-arm doors closed. Five reinforcers (Froot Loops; Kellogg Co., Battle Creek, MI) were randomly placed in the circular cylinder, and each rat was given a maximum of 5 min to consume all pieces.

On PND36, training was initiated and continued for 4 weeks with 4 days per week for a total of 16 sessions per rat. Training was conducted during the dark phase of the light cycle. Training was conducted using a working/reference memory paradigm (Brandeis et al., 1995; Daniel et al., 1997; Olton, 1987) in which 8 of the 12 arms were randomly selected and baited. Recessed food cups were placed at the end of each arm containing either bait or no bait. The same arms remained baited for the entire experiment. Each rat was placed in the central hub for 5 s to orient itself. The rats were allowed 5 min to explore and retrieve all eight reinforcers. The maze was cleaned after each training session to remove scent cues. Entry into an unbaited arm was scored as a reference memory error. Alternately, entry into a baited arm was scored as a correct choice and reentry was scored as a working memory error. Subsequently, working memory was evaluated using the total number of errors in the first eight arm visits. With respect to reference memory, these were calculated based on the number of entries into unbaited arms and presented as the number of reference memory errors. Response time (seconds per entry) was also calculated.

It is important to note that during the period of maze training, feed was restricted to provide sufficient motivation for the rats to explore the maze. However, since this is a period of rapid growth and high-energy demand, any energy imbalances could affect maze acquisition. Therefore, to prevent any potential energy imbalances that might occur from restricting the food of growing animals, we developed and used a decrement-feeding regimen which consisted of a gradual reduction in the amount of food given per week. Accordingly, rats were fed 95% during the first week and second week, 90% during the third week, and finally 85% during the fourth week of their free-feeding level to maintain a reasonable body weight gain.

**Statistical analysis.** Cholinesterase and physical development data were analyzed by ANOVA using the mixed-model procedure (Littell et al., 1996) of SAS (SAS Institute, 2008) followed by separation of means using least significant difference (LSD). Prior to analysis, values from pups of the same sex and same treatment within the same litter were averaged to obtain a single value for each time point. Body weights were log transformed and analyzed by ANOVA using the mixed-model procedure with a compound symmetry covariance structure and age as a repeated-measures factor. The timed reflex tests and maze data were analyzed by ANOVA using the mixed-model procedure with a first-order autoregressive covariance structure and either day (for the timed reflex tests) or week (for the maze data) as a repeated-measures factor. In all repeated-measures analyses, litter was included as a random effect and rat nested within treatment was included as subject in both the repeated statement and random statement. Analyses identified differences in week, sex, and treatment and all possible interactions followed by separation of means using LSD. The criterion for significance was set at $p < 0.05$.

**RESULTS**

No signs of overt functional toxicity due to OP treatment were observed in the rats. With respect to body weights (Figs. 1A and 2A), overall analysis demonstrated a significant effect of sex ($F_{1,1390} = 337.56, p < 0.0001$), treatment ($F_{5,139} = 2.71, p < 0.0162$), age ($F_{10,1390} = 28449.9, p < 0.0001$), and significant sex × age ($F_{10,1390} = 130.2, p < 0.0001$) and treatment × age ($F_{60,1390} = 2.75, p < 0.0001$) interactions. However, there were no significant sex × treatment × day or sex × treatment interactions. Lower level analyses of females and males separately demonstrated a significant treatment × age interaction in both females ($F_{60,666} = 2.21, p < 0.0001$) and males ($F_{60,730} = 1.30, p < 0.0493$). In females, a significant reduction in weight was observed with the high dosage of CPS on PND13 and PND21 but not at any other age. In males, significant reductions in weight were observed in high dosage of CPS on PND5 and in the medium and high dosages of both CPS and MPS on PND13 and PND21, but recovery to control levels had occurred by PND36. While body weight was decreased in many treatment groups throughout the experiment, there was only one age at which a statistically significant decrease in body weight was present. This occurred on PND46 in the medium CPS group, but there were no statistically significant differences prior to that age on PND43 or after that age on PND51. However, with respect to any other physical development parameter measured, there were no statistically significant effects of exposure on pinna detachment, downy fur development, hair growth, incisor eruption, and eye opening (data not shown). Similarly, with respect to reflex development, there were no statistically significant effects of exposure on surface righting, negative geotaxis, cliff avoidance, free fall righting, and acoustic startle (data not shown).

As described above, a decrement-feeding regimen was developed and used in this study which induced no significant weight loss as the percent free-feeding level was reduced. This method of food restriction is distinct from that commonly used with adults where weights are kept at approximately 85% of free-feeding levels. This different feeding schedule could have had an effect on the motivation to learn the task since the rate of learning was not as robust as was expected. Both control and...
OP-treated rats were subjected to identical procedures, so any differences between these groups would be expected to be the result of treatment. However, the average body weight of all treatment groups fell within the range of normal body weights for this strain of rats based on the purchasing information from the supplier.

With respect to ChE inhibition, the pattern was similar between males and females with no significant differences, and data were pooled for presentation (Fig. 2). On PND20, specific activity was significantly reduced with all dosages of both compounds. On PND30 and PND40, the CPS low-dosage group had returned to control levels, but ChE activity in all other treatment groups remained reduced. While both compounds persistently inhibited ChE, the low dosage of MPS was more persistent in comparison to the low dosage of CPS. By PND50, ChE activity was no longer significantly inhibited with either compound at any dosage.

With respect to response time (data not shown), the main effect of sex was significant ($F_{1,417} = 41.25, p \leq 0.0001$), and there was a significant sex $\times$ treatment interaction ($F_{6,417} = 2.33, p \leq 0.0314$) but no significant treatment effect or significant sex $\times$ treatment $\times$ week interaction. However, following mean separation, there were no significant differences between the control and treated groups in either sex, but the response time was significantly faster in females than in males in all treatments.

With respect to working memory, the parameter utilized was total number of errors in the first eight arm visits. This is presented as 4-day blocks over 16 days of training in Figures 3A and 3B for females and Figures 4A and 4B for males treated with CPS and MPS, respectively. Overall, sex ($F_{1,457} = 43.49, p \leq 0.0001$), treatment ($F_{6,99} = 4.95, p \leq 0.0002$), and week ($F_{3,457} = 10.24, p \leq 0.0001$) were significant, and there were significant sex $\times$ treatment ($F_{3,457} = 4.32, p \leq 0.0051$) and sex $\times$ treatment $\times$ block ($F_{18,457} = 1.71, p \leq 0.0344$) interactions. In the controls, males appeared to learn the maze faster than females with a significant decrease in working memory errors between the first and second week in males while significant improvement did not occur in females until between the second and third week. With respect to treatment, there was no effect on working memory between the control and treated females during any week of testing. In males, a greater number of errors were observed with the high dosage of CPS during all 4 weeks of testing and with the low and medium dosages of CPS during the fourth week. A greater number of errors were observed with the medium dosage of MPS during the first, third, and fourth weeks and with the high dosage of MPS during the second, third, and fourth weeks. To determine overall treatment effects, lower level analyses were performed and the average number of errors in the first eight arm visits over 16 days of training is presented for females.
In females, there were no cumulative effects, but in males, performance was decreased with all treatment groups with statistical significance occurring in the medium and high dosages of CPS and MPS. With respect to sex differences, there were no significant differences between control males and females, but sex differences were apparent in the medium and high dosages of CPS and MPS.

Reference errors are presented as 4-day blocks over 16 days of training in Figures 5A and 5B for females and Figures 6A and 6B for males treated with CPS and MPS, respectively. With respect to reference memory, sex was not significant, while treatment ($F_{6,99} = 3.64, p < 0.0027$) and week ($F_{3,457} = 14.00, p < 0.0001$) were. There were significant sex $\times$ treatment ($F_{6,457} = 3.66, p \leq 0.0015$) and sex $\times$ treatment $\times$ week ($F_{18,457} = 1.91, p \leq 0.0138$) interactions. As with working memory, there was a significant decrease in reference errors between the first and second week in males while significant improvement did not occur in females until between the second and third week. In females, significantly fewer reference errors were made during the second week in the medium and high CPS groups and during fourth week in the medium CPS group. No significant effects were observed with MPS. In males, a significant increase in the number of reference errors made was observed during the second week with the medium and high dosages of CPS. With MPS, increased numbers of reference errors were observed during the second and third weeks and with the low and high dosage of MPS and during the third and fourth weeks with the medium dosage of MPS. To determine overall treatment effects, lower level analyses were performed and the average number of reference errors over 16 days of training is presented for females (Fig. 5C) and males (Fig. 6C). In females, the medium and high dosages of CPS significantly reduced the number of total reference errors made while, in males, the number of reference errors was significantly increased by the high dosage of CPS and with all dosages of MPS. With respect to sex differences, the number of reference errors was significantly different in the control groups and with the low and high dosage of CPS.

DISCUSSION

The present study sought to control the rearing environment by including multiple treatment groups within each litter. It is known that variations in maternal care within the same strain of rats can lead to differences in behavioral performance including spatial memory (Barha et al., 2007; Caldji et al., 1998; Zhang et al., 2005). The study was designed to take advantage of the litter effect (i.e., the tendency for littermates all to respond in the same way to stimuli) to insure that effects are due to treatment and not litter differences. However, this design does not control for differential maternal responsiveness to treated compared with control offspring (Booze and Mactutus, 1985;
Ruppert et al., 1983). It is unknown whether CPS or MPS induces differential maternal responses. However, the CPS-induced spatial learning deficits observed here are similar to those reported by Levin et al. (2001), who used a between-litter design, which suggests that maternal factors do not significantly alter the effects of CPS on brain development. This design also does not control for variations in nursing between control and treated pups that could have contributed to our observed differences in body weights. Our data are in agreement with that of Song et al. (1997) who treated all rat pups in the same litter with 1 mg/kg CPS from PND1 to PND4 and observed no significant changes in body weight on PND5. Similarly, we did not see any significant decrease in body weight on PND5 using the same dosage. However, we did observe that a higher dosage of CPS (1.5 mg/kg) significantly decreased body weight by 14%. It is not clear if this difference is due to differences in competition for nursing sites between treatments or is due to the physiological effects of CPS on the pups in the highest dosage group.

Environmental exposure to OP insecticides is suspected to affect cognitive function, neuromuscular integrity, and to induce neurological deficits in children (Guillette 2000; Guillette et al., 1998; Rauh et al., 2006; Ruckart et al., 2004). In this study, early postnatal repeated exposure to dosages of CPS or MPS which exhibited no overt signs of toxicity produced neurocognitive changes in visuospatial learning and memory formation during the period of the juvenile and adolescent stages of development in rats. This exposure paradigm changed the normal behavioral performance even during the period when ChE was not inhibited, suggesting that the temporary increase in ACh following early postnatal ChE inhibition could result in long-term effects which may reflect the alteration of other molecular targets. Alternatively, the early postnatal exposure paradigm may have simply disrupted the normal cholinergic neuronal development leading to behavioral deficits (Gupta et al., 1985).

It has been reported that males inherently perform better on spatial memory tasks than do females (Brandeis et al., 1989; McNamara and Skelton, 1993; Vorhees et al., 2004). Our data suggest that overall performance did not differ between control males and control females since both made a similar number of errors within the first eight-arms visits. However, our data do somewhat agree with this line of thought in that male controls made more improvement earlier in the behavioral testing than did control females. This difference may be related to response time since the response time in females was significantly faster than that in control males. In other words, females moved much faster when trying to acquire each reinforcer, and this may have played a role in the number of increased errors. In addition, control males committed fewer reference memory errors than did control females.

The acquisition of the radial arm maze by males but not females was reduced by exposure to both CPS and MPS. Even the lowest dosages of MPS and CPS which did not result in
systemic toxicity (i.e., reduced body weight) demonstrated decrease performance in spatial acquisition. In contrast, exposure of females had little effect. This sex selectivity has previously been demonstrated in rats exposed postnatally to CPS (Levin et al., 2001) during the critical window for sexual differentiation (Dam et al., 2000, Hohmann 2001; Pryor et al., 2000; Roselli and Klosterman, 1989).

In males, MPS appeared to produce greater deficits in working memory than did CPS. This was especially evident with the medium-dosage MPS treatment group which performed at a significantly lower level than did the medium-dosage CPS treatment group. This may suggest that developmental exposure to MPS induces more damage to the mechanisms responsible for short-term memory function than does developmental exposure to CPS. It also must be stated that MPS (LD₅₀ 14–25 mg/kg) is inherently more toxic than CPS (LD₅₀ 82–245 mg/kg) and could possibly result in greater cell damage. However, while significant effort was made to use dosages that resulted in similar levels of brain ChE inhibition, MPS has a faster time to peak inhibition in vivo than does CPS (Chambers and Carr, 1993). This faster pattern of cholinesterase inhibition could result in a more rapid impact on the cholinergic system. However, recovery from inhibition occurs faster following with MPS exposure than it does following CPS exposure which suggests that the effects of CPS is more persistent. The basis for these differences in performance deficits in males is not clear. While it has been previously reported that exposure of rats to CPS during the early postnatal period selectively impairs working memory performance of males (Levin et al., 2001), this is the first report demonstrating the impairment of working memory and reference memory following incremental developmental exposure of MPS. In addition, this study demonstrated that neurobehavioral differences exist following developmental exposure to CPS and MPS. This is in line with other studies which have reported similar findings between other OPs including CPS, diazinon, and parathion (Levin et al., 2001, Timofeeva et al. 2008a,b).

CPS and MPS not only altered short-term memory but also long-term memory, a process that is known to require synthesis of new proteins and strengthening of neuronal connections (Ashraf et al., 2006; Alkon et al., 2005). This study also demonstrates that these differences are sex selective. In males, all three dosages of MPS increased the number of reference errors, while with CPS, only the high dosage had significant effects. In contrast, MPS had little effect in females but the medium and high dosages of CPS actually reduced the number of reference errors. Sex-selective changes in long-term memory in the radial arm maze following developmental exposure to CPS have been previously reported (Levin et al., 2001). Here we report that MPS produces similar sex-selective changes

**FIG. 5.** Reference errors in female rats exposed developmentally to three incremental dosages of either CPS or MPS for 21 days as described in the ‘‘Materials and Methods’’ section. (A and B) Mean number of reference errors (± SE) presented as 4-day weeks over 16 days of training for (A) CPS and (B) MPS. An asterisk denotes a significant difference from control within that week. (C) Mean number of reference errors (± SE) averaged over 16 days of training (n = 9–14). Bars with different lower case letters are significantly different from one another (p < 0.05). A dagger denotes a significant difference from males for that specific treatment group.
which appear to be more dramatic. Although in vitro studies have shown a weak relationship between CPS and estrogenic activity and between MPS and inhibition of androgen receptor activity (Andersen et al., 2002; Kojima et al., 2004), it is still unclear if the enhancements observed in females as opposed to the deficits observed in males are related to disruption of estrogenic activity or disruption in brain sexual differentiation.

It is known that memory impairment is produced after acute accidental exposure to OPs in humans (Dharmani and Jaga, 2005). In these acute human cases, the increased inhibition of AChE produced confusion and memory deficits, presumably due to overstimulation of postsynaptic receptors. Conversely, in chronic exposure scenarios which are more indicative of human exposures, very limited epidemiological association exists. Our previous studies have shown significant inhibition of ChE and compensatory reduction in muscarinic receptor densities during the period of repeated CPS or MPS exposure (Guo-Ross et al., 2007; Tang et al., 1999, 2003). In this study, significant ChE inhibition was present up to 19 days after cessation of exposure. As stated earlier, disruption of cholinergic and non-cholinergic synaptic components have been reported following developmental CPS exposure. However, none of these effects, that is, receptor levels or synaptic components, directly parallel our behavioral findings. They do suggest that developmental OP exposure has the potential to disrupt synaptic function, and this disruption is present during the period where we observed behavioral alterations. It has been previously suggested that OP exposure may change neuronal connectivity in the developing brain which could result in aberrant behavioral outcomes (Bigbee et al., 1999; Brimijoin and Koenigsberger, 1999).

In this study, similar levels of ChE inhibition were observed between the two compounds but the compounds induced different behavioral effects. It could be concluded that the behavioral effects are not related to the cholinergic effects but are related to unknown non-cholinergic mechanisms. However, it is known that during the period of developmental exposure, the recovery pattern of ChE activity differs between MPS and CPS with faster recovery following MPS than CPS (Tang et al., 2003). Thus, our single time-point measurement of ChE activity may not have captured the time of peak inhibition thus it may not be a true reflection of the level of inhibition that was actually present. In addition, the physiological compensation to the increased ChE inhibition differs between MPS and CPS (Tang et al., 1999, 2003). Thus, the different behavioral effects observed between MPS and CPS may be the result of compound differences in the toxicodynamics in the brain during the exposure. Therefore, at this juncture, it cannot be concluded that the non-cholinergic mechanisms are totally responsible for the behavioral outcomes.

In conclusion, solving a behavioral maze is seldom based on a single memory system and requires interactions between

FIG. 6. Reference errors in male rats exposed developmentally to three incremental dosages of either CPS or MPS for 21 days as described in the “Materials and Methods” section. (A and B) Mean number of reference errors (± SE) presented as 4-day weeks over 16 days of training for (A) CPS and (B) MPS. An asterisk denotes a significant difference from control within that week. (C) Mean number of reference errors (± SE) averaged over 16 days of training (n = 9–14). Bars with different lower case letters are significantly different from one another (p < 0.05). A dagger denotes a significant difference from females for that specific treatment group.
different memory systems to correctly learn the task. In many instances, damage to one system can have important consequences for learning dependent on another system and lesions of one system often enhance learning of tasks associated with another system (Gold, 2003). Thus, the mechanisms by which developmental OP exposure induces aberrant behavior may include dysfunction of both cholinergic and non-cholinergic systems, and it is possible that these dysfunctions do not occur simultaneously. It is currently unclear if the dysfunction of the non-cholinergic systems is the result of direct effects on the cholinergic system that occur during exposure or is the dysfunction the result of direct effects on distinct molecular components of the non-cholinergic systems themselves.

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