Long-Term Neurotoxicity of Chlorpyrifos: Spatial Learning Impairment on Repeated Acquisition in a Water Maze

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INTRODUCTION

Organophosphate (OP) compounds are chemicals widely used as insecticides and as industrial additives and chemical warfare agents. The primary mechanism of acute toxic action is the inhibition of acetylcholinesterase (AChE) (Ecobichon, 1997; Lotti, 2001). When AChE is inhibited, the level of acetylcholine rises in the synaptic cleft, producing both nicotinic and muscarinic symptoms, as well as signs of intoxication in the peripheral and central nervous systems (Namba et al., 1971). Moreover, Casida and Quistad (2004) and others (Pope, 1999) have pointed out the existence of non-AChE targets.

Some long-term neurotoxic consequences of OP exposure are well established (reviewed in Ehrich and Jortner, 2001), but others are still the subject of debate and active research. An organophosphorus ester–induced chronic neurotoxicity (OPICN) syndrome has been proposed (Abou-Donia, 2003; Ahmed and Davies, 1997; Colosio et al., 2003). The OPICN syndrome could result from long-term exposure to subclinical doses of OP (reviewed in Abou-Donia, 2003; Jamal, 1995, 1997; Jamal et al., 2002; Lotti, 2001). Data on humans who have suffered from acute OP poisoning also show alterations in neuropsychological performance. Kaplan and collaborators (1993) reported memory impairment and intellectual functioning decline months after acute exposure to chlorpyrifos (CPF). Agricultural workers tested about 2 years after a pesticide poisoning episode showed significantly lower performance in verbal and visual attention, visual memory, visuomotor speed, sequencing, and problem solving (Rosenstock et al., 1991). Similar results have also been found in agricultural settings (Savage et al., 1988; Steenland et al., 1994). Some 6 to 8 months after the terrorist attack with the chemical weapon sarin gas in the Tokyo subway, some neuropsychological and brain evoked potential deficits were found to remain (Yokoyama et al., 1998a, 1998b). Altogether, these data point to action by OPs, either as pesticides or as nerve agents, as being responsible for the long-term functional neurotoxicology.

Studies in which animals are used as experimental subjects have also shown the cognitive and behavioral effects of OPs. These studies may also be divided into those that focused on the effects of acute doses of OPs (Bushnell et al., 1993, 2001; Levin et al., 1995; Sánchez-Amate et al., 2001) and those that focused on chronic or repeated exposure (Bushnell et al., 1991, 1994; Cohn and MacPhail, 1997; Prendergast et al., 1997, 1998; Stone et al., 2000; Upchurch and Wehner, 1987; but see Maurissen et al., 2000).

The above-mentioned studies focus on the immediate effects of either acute or chronic exposure to OPs. Nevertheless, there are a limited number of studies that assess the persistent...
consequences in animals, lasting long after OP exposure has ended. Because data from human subjects point to the neuro-psychological deficit after acute OP intoxication, the development of animal models for study of the cognitive decline is required. In this context, some studies have found cognitive deficits lasting days or even weeks after cessation of OP exposure (Kassa et al., 2001a; Raveh et al., 2002). Duffy and Burchfiel (1980) reported long-term effects on EEG in *Macaca mulatta* when evaluated 1 year after sarin exposure.

Recently, we have found performance decrements in a four-trial repeated acquisition spatial task in a water maze (Sánchez-Santed et al., 2004). Animals were treated twice, at 16 and 38 weeks of age, with an acute injection of CPF or paraoxon. When evaluated 22 weeks after the second injection, the CPF-treated animals showed difficulties in finding the platform in a new position on each first daily trial. However, performance on trials 2 to 4 did not differ from controls. This effect was especially evident once groups reached asymptotic performance.

The present work was therefore undertaken to extend the long-term behavioral effects observed. To determine whether two distant exposures are necessarily required to induce such effects, two experiments were designed. Rats were injected either once or twice with CPF and then tested 21 or 26 weeks after the treatment was ended. To accomplish the behavioral assessment, performance in a two-trial repeated acquisition task in the water maze was examined. This behavioral test has frequently been employed to analyze spatial memory as well as the accuracy of animals’ search relative to the escape platform location each new session (Gallagher et al., 1993; Roloff et al., 2002a, 2002b; Steele and Morris, 1999; Van Groen et al., 2002).

In addition, parallel groups of rats were included to determine neurobehavioral effects and brain AChE activity after the dose of CPF employed. Subcutaneous administration of CPF (250 mg/kg) produces a high reduction of brain AChE activity (Sánchez-Amate et al., 2001; Sánchez-Santed et al., 2004). This dose is lower than the maximally tolerated dose (MTD) for which clinical signs of cholinergic overstimulation are absent in animals exposed to CPF (Pope et al., 1992).

**MATERIALS AND METHODS**

**Animals.** Twenty-six naive male Wistar albino rats were purchased from the animal facilities at the University of Granada. They were 4 months old at the beginning of the experiment, with a weight range from 270 to 410 g. Groups of four rats were housed in an environmentally controlled room (22°C temperature with an 8:00 h/20:00 h light/dark cycle) in the animal laboratory of the Departamento de Neurociencia y Ciencias de la Salud of the Universidad de Almería. Food and water were continuously available in the home cages. Eight additional rats matched in sex, age, weight, and housing conditions were used as parallel groups to determine neurobehavioral toxicity and brain AChE activity after OP administration. All procedures were performed in accordance with Spanish Royal Decree 223/1988 on the protection of experimental animals.

**Apparatus.** Water maze testing was performed in a circular black plastic pool (height: 50 cm, diameter: 150 cm). The tank was filled with clear water kept at 22°C. Twelve black platform holders were attached to the floor of the pool, forming a concentric circle of 12 different positions at 30 cm from the maze wall. During the spatial task, a black escape platform (height: 38.5 cm, diameter: 10 cm) was submerged 1 cm below the surface of the water. The rats could not see the black platform against the black pool because of the lighting. For the visual task, a white escape platform with the upper 1.5 cm above the surface of the water was used. Platforms were located in 1 of the 12 different possible positions. Four starting positions were marked on the outer wall of the maze (N: north, E: east, S: south, and W: west). Swimming activity of each rat was monitored by a small video camera 150 cm above the center of the pool. The camera was connected to a video recorder. For AChE activity determination a DU 530 Beckman spectrophotometer was used.

**Toxicity evaluation.** Eight rats were divided in two groups (n = 4) depending on treatment: a CPF group, 250 mg/kg of CPF; a VHC group, olive oil. Chlorpyrifos was dissolved in olive oil and 1 ml/kg was administrated by subcutaneous injection. At both 24 h and 48 h after dosing, a short version of a functional observational battery was used to evaluate neurobehavioural toxicity (Moser, 1995; Sánchez-Amate et al., 2001, 2002).

**AChE determination.** Immediately after toxicity evaluation, animals from the parallel group were decapitated. The whole brain was removed and immediately homogenized with 1% Triton X-100 in 0.1 M sodium phosphate buffer at pH 8 at a ratio of 1/10 (w/v). The homogenate was centrifuged at 10000 × g for 10 min. The pellet was discarded and the supernatant was kept for AChE assay. Cholinesterase activity was determined spectrophotometrically by the Ellman method (Ellman et al., 1961) using acetylthiocholine iodide (30 μl; final concentration = 0.5 mM) as substrate and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (200 μl; final concentration = 0.33 mM). Assay tubes were completed to 1 ml with Na phosphate buffer, pH 8. Control measurement was done by adding a specific inhibitor of AChE, BW284C51 (10 μl; final concentration = 10 μM). Enzyme activity was calculated relative to protein concentration by the Bradford method (Bradford, 1976).

**Experiment 1**

**Organophosphate administration.** Fourteen rats were divided into two groups (n = 7). Animals were treated twice at 16 and 37 weeks of age. The CPF1 group received two doses of 250 mg/kg chlorpyrifos (Riedel-de Häén, purity > 99.5); the VHC, group received the olive oil form: chlorpyrifos was dissolved in olive oil and 1 ml/kg was administrated by subcutaneous injection.

**Spatial task.** Behavioral assessment was carried out 21 weeks after the last CPF injection. Each rat performed two spatial task trials per day in 40 consecutive sessions. In each session, two different starting positions were used. The hidden escape platform location was changed for each session using the 12 platform holders in a pseudorandom sequence; within each session, the position remained the same. For each trial, the rat was lowered into the water facing the wall. Rats were allowed to swim for a maximum of 90 s so they could find the platform. Once this time had elapsed, if a rat still had not found the platform, it was guided by hand onto the platform and allowed to spend 15 s there. When the animal’s performance was successful and it found the platform by itself before the 90 s period had elapsed, it was allowed to stay on the platform for 15 s. During the intertrial interval (30 s) animals were removed to a waiting cage until the next trial.

After the last session (session 40) an additional trial (Trial 3; transfer test) was given, in which the platform was removed from the pool and rats were allowed to swim for 90 s. To assess spatial bias, the rats’ performance was recorded on video tape for later analysis. An observer blind to the experimental conditions measured the time spent in each of the four previously designated quadrants: The quadrant where the platform was located during the prior to trials (P), the opposite quadrant (O), and the left and right quadrants (L and R, respectively). Spatial bias was assessed by registering the time spent in each of the four quadrants during the first 10 s of the trial, as well as during the whole transfer trial.
**Visual task.** After the spatial task, four additional sessions were given to assess the animals’ ability to find a visible platform. Those sessions were identical to the above-described spatial task, except that the visible white platform was used.

**Experiment 2**

**Organophosphate administration.** Twelve rats were divided into two groups \( n = 6 \). Animals were treated once at 16 weeks of age. CPF\(_2\) group: 250 mg/kg CPF; VHC\(_2\) group: olive oil; chlorpyrifos was dissolved in olive oil and 1 ml/kg was administered by subcutaneous injection.

**Spatial/visual task.** Behavioral assessment was carried out 26 weeks after CPF treatment. Sessions in both tasks were identical to the above-described experiment 1. Each rat performed two spatial task trials per day in 27 consecutive sessions. After those sessions, an additional session (session 28) was given (transfer test). Two trials identical to the above-described trial for the spatial task were given, except for trial 2, in which the platform was removed from the pool and rats were allowed to swim for 90 s. Spatial bias was assessed as described for experiment 1, registering the time spent in each of the four quadrants during the first 10 s of the trial as well as during the whole transfer trial.

After the twenty-eighth session, three new sessions were given (visual task) to assess the animals’ ability to find a visible platform. Those sessions were identical to the above-described visual task (Experiment 1).

**AChE determination.** Once the behavioral assessment was performed, animals from both experiments were decapitated and whole brain was removed for AChE determination (see above).

**Data analysis.** Statements of statistical significance are based on a repeated-measures analysis of variance (ANOVA). The Newman-Keuls test was used for post hoc analysis. Student’s t-tests were used for between-group comparisons in the AChE study. The accepted level of significance for all tests was \( p \leq 0.05 \).

**RESULTS**

**Neurobehavioral Battery and Brain AChE Activity**

Overall, the dose of CPF was well tolerated. Clear signs of acute toxicity were detected. Treated animals showed, at 24 and 48 hours, weight loss and body temperature reduction. Twenty-four hours after injection, depressed activity in the home cage was also observed: Activity measures in the home cage were lowered in CPF-treated animals compared to controls. No other signs of acute cholinergic toxicity were detected. However, whole brain AChE activity was 4.63% (S.E.M. 0.96) for the CPF group compared to vehicle-treated animals \([t(6) = 8.616; p < 0.001]\).

**Experiment 1**

During the 21-week period, one animal in CPF\(_1\) group became ill and was removed from the experiment. (CPF\(_1\), \( n = 6 \); VHC\(_1\), \( n = 7 \)). Figure 1 (panels A and B) summarizes the results obtained during the repeated acquisition task in the water maze. This phase of the experiment was performed 21 weeks after treatment was ended. Sessions were collapsed for statistical analysis in blocks of 2 sessions. Repeated measures analysis was fulfilled by using group (VHC, CPF) as a between-subjects factor, and by using two within-subjects factors (blocks of sessions and trials, trials with 20 and 2 levels, respectively). Rats in each group demonstrated the ability to learn the task. A main effect of block of sessions \([F(19,209) = 9.945; p < 0.001]\) and trial \([F(1,11) = 12.425; p < 0.01]\) was observed. However, a group by blocks of sessions interaction was significant \([F(19,209) = 1.702; p < 0.05]\). The two groups behaved in a different way across blocks of sessions.

The analysis of the interaction was carried out by analyzing group latencies across blocks of sessions. Moreover, as in a prior study, blocks of sessions were divided between the acquisition and performance phases, in function of the performance of the control animals (Sánchez-Santed et al., 2004). The first 7 blocks were considered the acquisition phase, (VHC: main blocks of sessions effect significant), and the remaining 13 blocks were considered the performance phase.

![FIG. 1. Experiment 1. Mean (± S.E.M.) escape latency in the CPF\(_1\) and VHC\(_1\) groups during the repeated acquisition task in the Morris water maze. Each point represents the mean latency (A) for each block of two sessions, (B) for each trial. Broken line cuts sessions into acquisition and performance phases.](https://doi.org/10.1038/nn0202-0946)
(VHC: main blocks of sessions effect not significant). Repeated measures analysis was performed by using Group (VHC, CPF) as a between-subjects factor, and by using two within-subjects factors (trials: 2 levels, and blocks of sessions: 7 and 13 levels for the acquisition and performance phases, respectively). Rats in each treatment group similarly improved their latencies during the acquisition phase, as only a main effect of blocks was observed \[F(6,66) = 8.407; p < 0.001\]. Once in the performance phase, a main effect of blocks \[F(12,132) = 2.034; p < 0.05\] was still observed. However, group by blocks of sessions was significant \[F(12,132) = 2.034; p < 0.05\]. Both groups behaved in a different way across blocks of sessions. Performance of oil-treated rats remained constant across sessions \[F(12,72) = 1.460; p = 0.160\]. Organophosphate-treated rats differed from controls: A main effect of session was still observable \[F(12,60) = 3.308; p < 0.001\].

Figure 2 (panel A) shows the results of the transfer test. Repeated measures analysis was performed by using Group (VHC, CPF) as a between-subjects factor, and by using a within-subject factor (Position, four levels). A main effect of position was observed \[F(3,33) = 6.795; p < 0.01\] regardless of treatment group. Post hoc analysis indicated that animals preferably swam in the quadrant where the platform was previously placed. Results from the analysis of the first 10 seconds of the transfer test are shown in Figure 2 (panel B). The Student t-test showed that both VHC\(_1\) and CPF\(_1\) groups swam the same amount of time in the quadrant of the platform.

Organophosphate-treated rats did not differ from controls on the visual task (data not shown). Whole-brain AChE activity was 0.0447 and 0.0378 \(\mu\)mol mg\(^{-1}\) min\(^{-1}\), determined after behavioral assessment for VHC\(_1\) and CPF\(_1\) groups, respectively. Student’s t-test did not show any difference between groups \[t(11) = 1.816; p = 0.097\].

**Experiment 2**

The repeated acquisition task in the water maze was performed 26 weeks after CPF or olive oil injection. Figure 3 summarizes results for this phase of the experiment. Sessions were collapsed for statistical analysis in blocks of 2 sessions. Repeated measures analysis was performed by using Group (VHC, CPF) as a between-subjects factor, and by using two within-subjects factors (blocks of sessions and trial, with 14 and 2 levels, respectively). A main effect of block \[F(13,130) = 10.432; p < 0.001\] and trial \[F(1,10) = 19.117; p < 0.001\] was observed. Rats in both groups demonstrated a performance improvement across blocks of sessions (Fig. 3, panel A). However, a group by trial interaction was significant \[F(1,10) = 4.806; p = 0.05\].

Post hoc analysis indicated that in trial 1, the ability to find the hidden platform was practically identical in CPF\(_2\) and VHC\(_2\) groups. Nevertheless, animals that had received CPF showed impaired performance during the second trial compared to animals that had received olive oil (Fig. 3, panel B). Animals from the VHC group showed a reduction in their escape latencies in trial 2 with respect to trial 1 \(p < 0.01\). Chlorpyrifos-treated animals performed both trials with comparable escape latencies \(p = 0.195\).

Figure 4 (panel A) shows the results from the spatial bias test. Repeated measures analysis was performed by using Group (VHC, CPF) as a between-subjects factor, and by using a within-subject factor (Position, four levels). A main effect of position was observed \[F(3,30) = 14.595; p < 0.001\]. Post hoc analysis showed that animals preferably swam in the quadrant where the platform was previously placed, as well as the one where the rats were introduced into the pool (quadrant O) \(p < 0.05\). When analyzing the first 10 s of the transfer test (Fig. 4, panel B), the Student’s t-test showed that VHC animals swam for a longer time in the quadrant of the platform than did CPF-treated animals \[t(10) = 3.993; p < 0.01\]. After the spatial bias test, the VHC\(_2\) and CPF\(_2\) groups were assessed in a visual task.
Animals from both groups showed similar performance in visual acuity. Finally, once the behavioral assessment was ended, whole-brain AChE activity was determined. Enzymatic activity was 0.0436 and 0.0390 l mol mg⁻¹/min⁻¹ for the VHC₂ and CPF₂ groups, respectively. Student’s t-test did not show any difference between groups \( t(10) = 0.713; p = 0.492 \).

**DISCUSSION**

The present data confirm and extend the findings of long-term behavioral effects of acute subcutaneous administration of CPF in rats (Sánchez-Santed et al., 2004). As expected, 250 mg/kg CPF maximally reduced brain AChE activity to less than 5% of control. Weight loss, body temperature reduction and hypoactivity were the acute signs of intoxication observed. These results are similar to those reported in the literature, where the main acute effects found were weight and body temperature affectation (Chakraborti et al., 1993; Gordon and Grantham, 1999; Liu and Pope, 1996, 1998; Sánchez-Amate et al., 2001). Other clinical signs of cholinergic overstimulation were absent, proving that the dose used was lower than the MTD (Pope et al., 1992). The outcomes show that our treatment was as effective as intended in terms of acute neurotoxicity and enzyme inhibition.

Previously, we found long-term neurocognitive sequelae in animals treated twice with acute injections of CPF (Sánchez-Santed et al., 2004). Rats were injected at the age of 16 and 38 weeks with 166 and 250 mg/kg CPF, respectively. Twenty-two weeks after the second OP administration, performance in a four-trial repeated acquisition spatial task was shown to be impaired. Chlorpyrifos-treated animals took longer than VHC-treated animals to find the hidden platform on each first
daily trial. However, when a transfer test was performed, both groups showed a similar spatial bias. In the present study’s Experiment 1, rats were again treated twice with CPF, but this time with two identical doses of 250 mg/kg. Twenty-one weeks afterwards, treatment performance was assessed in a two-trial repeated acquisition task in a water maze. Rats in the CPF group demonstrated the capability to learn the task, but they took longer than the VHC-group to reach asymptotic performance. Again, in the transfer test, both VHC- and CPF-treated animals showed spatial bias, that is, the rats searched the area where the platform had been in the two immediately previous trials, regardless of treatment group.

Despite the long time between treatment and behavioral evaluation, and even after different doses of treatment and varying behavioral assessment procedures, CPF is capable of producing long-term deficits. We previously proposed that CPF-treated animals might have used a non-spatial search strategy to find the hidden platform during performance of trial 1, which would result in the pattern observed. This pattern included the above-mentioned first-trial performance impairment, but CPF-treated animals behaved like control animals in trials 2 to 4, as well as during the transfer test (Sánchez-Santed et al., 2004). Together, it seems that spatial learning was not totally impaired by CPF. However, in our experiment 1, where both doses were of 250 mg/kg, the spatial learning deficit looks different. CPF-treated group takes longer to reach the asymptotic performance during the repeated acquisition task. Rats previously injected with CPF showed a reduced performance compared to VHC-treated animals. Nevertheless, during the visual task, no differences were found between groups, suggesting that no gross motor, visual, or motivational variables are responsible for the effects. We hypothesize that CPF-treated animals are using a non-spatial search strategy to solve the task. In fact, the circling strategy has been proposed to be less effective but able to improve animal performance in the water maze task (Day and Schallert, 1996; D’Hooge and De Deyn, 2001; Gallagher et al., 1993). At present we are executing further experiments including more sophisticated technology (e.g., video-tracking systems). These could provide additional information to evaluate the reliability of these results and to discern the kind of strategy involved in solving the repeated acquisition task. Finally, we must take into account that during the transfer test, treated animals, as well as controls, showed a biased search to the quadrant associated with the platform. Note that the transfer test was done after forty sessions of the spatial task, once escape latencies of the treated-group approached escape latencies of controls. In this way treated rats are eventually able to learn about the platform location, despite the fact that they take longer to perform at control level in the repeated acquisition task.

The second aim of the present work was to determine if two exposures to CPF are required to induce a long-lasting neuropsychological decline. Thus, Experiment 2 was undertaken to study whether a single exposure to CPF is able to produce long-term effects. Several reports have shown that acute low-level doses of CPF cause immediate behavioral consequences: CPF induced an anxiogenic-like response in the elevated plus-maze 48 h after subcutaneous injection (Sánchez-Amate et al., 2001). Sustained attention during a visual signal-detection task was also compromised when rats were injected with non-symptomatic doses of CPF (Bushnell et al., 2001). Bushnell and collaborators (1993) also reported impairment in an operant delayed matching-to-position task (DMTP). Matching accuracy was reduced up to three weeks after a single subacute injection of CPF; however, the effect was ephemeral, as it vanished long before AChE activity had recovered (Pope et al., 1992). In our study, animals were injected once with 250 mg/kg CPF, and performance in a two-trial repeated acquisition task was assessed 26 weeks later. Despite their performance improvement across sessions, CPF-treated animals showed large escape latencies in each second trial compared to controls; that is, animals from the VHC-group, but not from the CPF group, showed an improved performance in trial 2 with respect to trial 1. Even though CPF-injected animals performed as well as controls when spatial bias was analysed, animals treated with CPF showed less preference to the platform quadrant when spatial bias was analysed during the first 10 seconds of the transfer test. Remember that in experiment 2, the transfer test was executed after just a sample trial. Together, the data indicate that CPF induced damage in the ability to retain spatial information in working memory. Second-trial performance during the repeated acquisition task reflects the use of the information relative to the escape platform location acquired during trial 1 (Brandeis et al., 1989; Frick et al., 1995; Roloff et al., 2002a, 2002b; Steele and Morris, 1999; Van Groen et al., 2002).

To our knowledge, there is little evidence of long-term sequelae in animals treated with OPs. One study has reported long-term effects on electroencephalogram in *Macaca mulatta* when evaluated 1 year after acute sarin exposure (Duffy and Burchfield, 1980). Stamper and collaborators (1988) found working memory deficits in radial arm maze performance of mature rats, in animals postnatally treated with repeated administration of parathion. These results suggest a selective spatial working memory deficit. This explanation is widely supported by several studies investigating short-term effects of the repeated exposure to low doses of OPs. Organophosphates produce lasting memory decline in animals performing a wide number of tasks. These effects have been registered both when animals are kept treated (Castillo et al., 2002; Llorens et al., 1993; Upchuch and Wehner, 1987) and after treatment (McDonald et al., 1988; Prendergast et al., 1997; Stone et al., 2000). For example, both chronic diisopropylfluorophosphate and CPF treatment reduced matching accuracy in a DMTP (Bushnell et al., 1991, 1994). Diisopropylfluorophosphate impairs learning, but not performance of a previously learned spatial navigation task (Prendergast et al., 1998; Stone et al., 2000; Upchuch and Wehner, 1987). Furthermore, a multiple schedule of repeated acquisition and performance
has shown that low doses of CPF decreased accuracy in repeated acquisition more than in performance (Cohn and MacPhail, 1997). It can be concluded that working memory was impaired; consequently, the learning of a new task or response sequence was affected, whereas performance of a familiar one was not. Moreover, the repeated acquisition procedure in the water maze seems a sensitive task for studying the long-term effects of OP intoxication, or at least, of that exerted by CPF.

The epidemiological literature has shown that both acute and chronic exposure to OPs induced long-term neuropsychological sequelae (reviewed in Abou-Donia, 2003; Ahmed and Davies, 1997; Colosio et al., 2003). Humans who have suffered from OP-acute poisoning showed long-term functional neurotoxicology: peripheral and central neuropathy (Duffy and Burchfiel, 1980; Kaplan et al., 1993; Steenland et al., 1994), affective disorders (Savage et al., 1988; Stallones and Beseler, 2002; Steenland et al., 1994; Whorton and Obrinsky, 1983), and neurocognitive deficit (Kaplan et al., 1993; Rosenstock et al., 1991; Savage et al., 1988; Steenland et al., 1994; Yokoyama et al., 1998a, 1998b). More specifically, memory impairment is often reported long after acute poisoning with OPs (Kaplan et al., 1993; Rosenstock et al., 1991; Savage et al., 1988). As described above, cognitive and behavioural effects of OPs are also reported by studies in which animals are used as experimental subjects. Neurocognitive decline has been shown shortly after acute (Bushnell et al., 1993, 2001; Levin et al., 1995; Sánchez-Amate et al., 2001) or chronic-repeated administration of OPs (Cohn and MacPhail, 1997; Bushnell et al., 1991, 1994, Prendergast et al., 1997, 1998; Stone et al., 2000; Upchurch and Wehner, 1987). Nevertheless, some studies have reported cognitive deficits lasting days or even weeks after cessation of OP-exposure. One week after soman administration, spatial learning in the Morris water maze was affected. Treated animals did not show spatial bias to the platform quadrant. Learning deficits were also observed during the reversal task (Raveh et al., 2002). Data from the present work and others (Duffy and Burchfiel, 1980; Sánchez-Santed et al., 2004; Stamper et al., 1988) clearly point to long-term neurocognitive sequelae in animals treated with OPs. However, the deficits reported here cannot be related to the well-known neurochemical changes induced by subcutaneous CPF. AChE activity, as well as muscarinic receptor binding, recovered long before behavioural assessment was carried out. (Chakraborti et al., 1993; Pope et al., 1992). Further research would allow us to achieve additional information about cognitive alterations, as well as the dose-response relationship and the long-lasting neurochemical mechanisms which result in the behavioural changes reported here.

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