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Atropine counteracts the depressive-like behaviour elicited by acute exposure to commercial chlorpyrifos in rats

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ABSTRACT

Acute organophosphate (OP) poisoning induces well-known signs of toxicosis related to acetylcholinesterase (AChE) inhibition. However, the relationship between acute OP poisoning and the onset of psychiatric disorders remains unclear. Thus, we investigated behavioural and biochemical consequences of acute exposure to the OP chlorpyrifos in male rats and also the effectiveness of the antidotes atropine and pralidoxime on reversing these changes. A sub-lethal dose of commercial chlorpyrifos (20 mg/kg, i.p.) elicited signs of acute toxicosis during the first hours after its injection in rats. Twenty-four hours after treatment, this single dose of chlorpyrifos induced a depressive-like behaviour in the rat forced swimming test without impairing locomotor activity. At this time (24 h), chlorpyrifos decreased plasma butyrylcholinesterase (BChE) activity and hippocampal, striatal and prefrontal cortical AChE activity in rats. The behavioural and biochemical consequences of acute chlorpyrifos poisoning do not seem to be long lasting, since 30 days later they were absent. We evaluated whether these behavioural and biochemical consequences of acute chlorpyrifos treatment would be reversed by the antidotes atropine (10 mg/kg i.p.) and/or pralidoxime (40 mg/kg; i.p.) given 1 h after poisoning. Pralidoxime partially reactivated the AChE activity in the prefrontal cortex, but not in the hippocampus and striatum. Atropine attenuated the depressive-like behaviour induced by chlorpyrifos in rats. Our results suggest that acute chlorpyrifos poisoning induces a transient depressive-like behaviour possible related to hippocampal AChE inhibition. They suggest that treatment with atropine and pralidoxime seems to be insufficient to counteract all the effects of OP acute poisoning, at least in rats.

1. Introduction

Organophosphate (OP) compounds are a group of highly toxic chemicals that have been used widely as pesticides in agriculture, especially in low and middle developing countries. Accidental or intentional OP exposures are common causes of poisoning worldwide, affecting approximately one million patients each year (Eddleston et al., 2008; Gunnell et al., 2007; Yurumez et al., 2007). Occupational exposure is particularly worrying in developing countries where the OP compounds are easily available, poorly regulated and used frequently without personal protection equipment (Gunnell et al., 2007; Recena et al., 2006a, 2006b).

OP compounds cause acute toxicosis by inhibiting acetylcholinesterase (AChE), the enzyme that hydrolyses acetylcholine (ACh) in central and peripheral cholinergic synapses (Mileson et al., 1998). Inhibition of AChE leads to an excess of ACh that, in turn, activates muscarinic and nicotinic receptors. During acute poisoning, OP pesticides affect, beyond skeletal muscles, mostly the cardiovascular, respiratory and central nervous systems (CNS; Jokanović and Kosanović, 2010; Vale and Lotti, 2015).

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Some clinical studies and case series suggest that acute exposure to OP compounds in various circumstances increases the risk of depression, suicide, neuropsychological and cognitive impairment in humans (Beseler et al., 2006; Colosio et al., 2003; Jaga and Dharmani, 2007; London et al., 2005; Lyu et al., 2018; Rosenstock et al., 1991; Savage et al., 1988; Stallones and Beseler, 2002; Wesseling et al., 2002). However, these epidemiological studies present some significant limitations. For instance, most of them are retrospective studies with poor details about the circumstances of acute exposure to OP compounds and, also, AChE activity measurement or signs of cholinergic syndrome were not assessed. Additionally, as these studies usually evaluated farmers, it is virtually impossible to specify the contribution of acute *versus* chronic exposure to OP compounds over the risk of depression or suicide. Moreover, there are no studies published so far that followed up the mental health of patients who survived a single and serious OP poisoning. Therefore, despite the aforementioned studies, the straight relationship between acute OP pesticides poisoning and increased risk of suicide or mood disorders remains unclear. An interesting approach to answer this open question would be to perform prospective longitudinal studies to properly assess levels of OP exposure and to monitor environmental conditions. Another approach is to investigate the consequences of acute OP poisoning similar to suicide attempts in preclinical models. The main advantages of preclinical studies are OP dose control, fixed interval between intoxication and evaluation and the measurement of the brain AChE activity.

The main therapeutic strategy to address acute OP poisoning consists in administering: 1) atropine to antagonize central and peripheral muscarinic cholinergic receptors; and 2) an oxime, such as pralidoxime, to reactivate the AChE inhibited by OP (Jokanovic, 2009; Vale and Lotti, 2015). Although the use of atropine is well established, the effectiveness of oximes in preventing death or serious damage is still controversial (Buckley et al., 2011; Eddleston, 2018; Eddleston et al., 2009; Pawar et al., 2006). Indeed, a clinical evidence pointed that atropine, given alone, seemed to be as effective as atropine combined with pralidoxime in the treatment of acute OP poisoning (de Silva et al., 1992). Besides that, benefits of these antidotes to prevent the onset of psychiatry disorders induced by acute OP poisoning have not been investigated yet.

Among different types of OP pesticides, chlorpyrifos has been widely used due to its relatively low cost and high efficacy (Chen et al., 2014). Repeated exposure of rodents at different life stages to chlorpyrifos provoked behavioural impairments related to depression and anxiety (Aldridge et al., 2005; Braquenier et al., 2010; Chen et al., 2011; Chen et al., 2014; Silva et al., 2017). Only few studies have investigated anxiety effects of acute exposure to this OP (López-Crespo et al., 2007, 2009; Sánchez-Amate et al., 2001). Nevertheless, there are no studies that investigated effects of acute exposure to high sub-lethal doses of chlorpyrifos on depressive-like behaviours in adult rats. Another open question is if the behavioural changes induced by chlorpyrifos poisoning would be persistent. Some evidence have suggested that the cholinergic neuronal dysfunction caused by OP exposure may be associated with long-term emotional damage (Chen, 2012). Finally, some clinical studies have shown that the index of suicide using OP is greater in men than in women (Kumar et al., 2014; Lekei et al., 2014). In this way, we investigated whether acute and sub-lethal poisoning with a commercial formulation of chlorpyrifos would induce acute and long-lasting depressive-like behaviour in male rats.

Although the mechanisms underlying the depressive-like effect of acute OP poisoning have not been elucidated so far, one hypothesis involves the overstimulation of cholinergic receptors in brain areas related to depression. Some direct and indirect evidence support this hypothesis. For instance, acute poisoning by the OP diisopropylfluorophosphate (DFP) induced depressive-like effect whereas decreased hippocampal and cortical AChE (Wright et al., 2010). On the other hand, some muscarinic antagonists seem to induce antidepressant effects in rodents and humans (Drevets et al., 2013; Mancinelli et al., 1988; Witkin et al., 2014). Thus, we investigated if acute poisoning with chlorpyrifos would reduce AChE activity in brain areas related to depression that receive cholinergic inputs. Further, considering that there are no clinical or preclinical studies evaluating the effects of the antidotes atropine and pralidoxime on behavioural changes caused by acute OP poisoning, we assessed whether these drugs, alone or in combination, would reverse the acute behavioural impairment elicited by chlorpyrifos. Finally, we also evaluated if pralidoxime would be capable of re-establishing brain AChE activity inhibited by chlorpyrifos, since the distribution of oximes through the CNS is limited (Sakurada et al., 2003; Voicu et al., 2010a).

2. Materials and methods

2.1. Animals

Adult male Wistar rats (300–370 g) bred and housed in the animal facility of our university were used in the present experiments. Rats were kept in groups of five per cage in a temperature-controlled room (24 ± 2 °C) under a 12 h/12 h light/dark cycle (lights on at 7:00 AM) with standard chow and water available *ad libitum*. The experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Committee for the Ethical Use of Animals in scientific research (CEUA-UFES, 047/2014). The animals were randomly distributed to the different treatment conditions and a total of 224 were used. Details of number of animals by group were shown in Supplementary Table S1.

2.2. Drugs

A commercial formulation of Chlorpyrifos [0,0-diethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothiate; 48% m/v, plus inert ingredients, Lorsban] was purchased from Dow Agrosciences (São Paulo, SP, Brazil) and was administered by intraperitoneal (i.p.) route at doses of 20, 25 and 30 mg/kg. Sub-lethal doses of chlorpyrifos were chosen based on a previous study from our group that identified 30 mg/kg of chlorpyrifos as a dose that produced robust cholinergic signs without inducing lethality (maximum tolerated dose; Cunha et al., 2018). Atropine (10 mg/kg; i.p.) and pralidoxime (40 mg/kg; i.p.) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Doses of atropine and pralidoxime were chosen on the basis of a study carried out by Kose et al. (2009). All drugs, including chlorpyrifos, were promptly diluted in NaCl 0.9% (saline) and injected in a volume of 1 ml/kg. In a pilot study, when chlorpyrifos was administered by the oral route, we detected much variability in the signs of acute toxicosis and inhibition of butyrylcholinesterase (BChE) activity. We considered this factor a limitation that would make it difficult to allow evaluation of the effectiveness of the antidotes. Thus, the i.p. route was chosen to allow accurate and efficient delivery of chosen doses and to reduce exposure variability. All drug solutions were always freshly made and immediately used to avoid decomposition.

2.3. Experimental design and procedures

2.3.1. Experiment 1

We evaluated the effect of a single injection of chlorpyrifos on the depressive-like behaviour in rats. Three single doses of chlorpyrifos (20, 25 or 30 mg/kg) were compared to the control group (saline) in rats submitted to the forced swimming test (FST) 24 h post-poisoning (n = 60). Animals received an i.p. injection immediately after the pre-test session of the FST.

To address whether chlorpyrifos poisoning would change locomotion and interfere with interpretation of behavioural analyses at the FST, independent groups of animals (n = 40) were evaluated in the open field test 24 h after a single injection of chlorpyrifos (20, 25 or

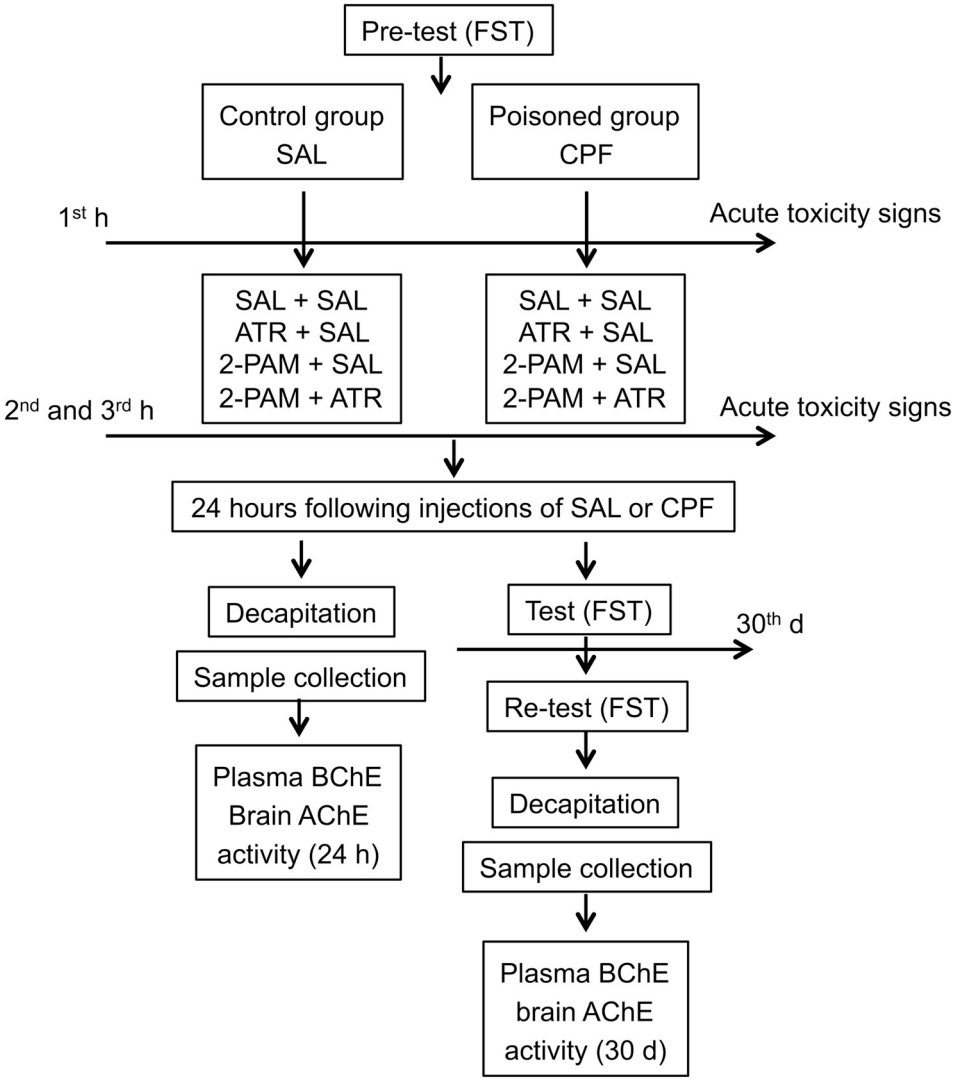

Fig. 1. Design of experiment 2. FST = forced swimming test; SAL = saline; CPF = chlorpyrifos; ATR = atropine; 2-PAM = pralidoxime.

30 mg/kg) to register the total number of line crossings, during 5 min.

2.3.2. Experiment 2

In this second experiment, we addressed if a single dose of chlorpyrifos would induce long-lasting depressive-like behaviour and also inhibit AChE in the hippocampus, prefrontal cortex and striatum of rats. To check if the chosen dose of chlorpyrifos (20 mg/kg) would lead to a mild poisoning, we also systematically recorded signs of acute toxicosis and plasma BChE activity. Furthermore, we treated additional groups of animals with atropine (ATR) and/or pralidoxime (2-PAM) to evaluate whether treatment with these drugs would prevent the onset of the depressive-like behaviour induced by the acute OP poisoning in the FST.

The experimental protocol is summarized in the Fig. 1. Immediately after the pre-test session in the FST, rats received a single injection (i.p.) of saline (SAL) or chlorpyrifos (CPF) and signs of acute toxicosis were scored. To mimic the starting time required for antidotes administration during OP poisoning in humans, 1 h later, the animals were treated with ATR or 2-PAM, solely or in combination (ATR plus 2-PAM). As a group of animals received both antidotes (ATR plus 2-PAM), all treated groups received a total of 3 injections (i.p.), as following described: SAL (SAL + SAL + SAL); ATR (SAL + ATR + SAL); 2-PAM (SAL + 2-PAM + SAL); ATR + 2-PAM (SAL + ATR + 2-PAM); CPF (CPF + SAL + SAL); CPF + ATR (CPF + ATR + SAL); CPF + 2-PAM (CPF + 2-PAM + SAL); CPF + ATR + 2-PAM (CPF + ATR + 2-PAM). All groups were evaluated to signs of acute toxicosis for up 2 h after the second set of injections. Twenty-four hours later, each group was divided in 2 additional subgroups: part of the animals (n = 44) were decapitated and blood and brain samples (hippocampus, striatum and prefrontal cortex) were collected, and the other part (n = 80) was submitted to the FST test 24 h (test session) and 30 days (re-test session) after poisoning. According to Mezadri et al. (2011), rats repeatedly exposed to the FST keep immobility behaviour constant. At the end of the re-test session, these animals were immediately decapitated and samples were collected to measure plasma BChE and brain AChE activity 30 days after the acute poisoning. The interval between treatment and test was chosen based on a previous study showing that 24 h after a single oral dose of chlorpyrifos, the spontaneous locomotor activity of the rats was recovered, but the hippocampal and cortical AChE activity was still inhibited (Nostrandt et al., 1997). The long-term evaluation 30 days after poisoning was designed based on the study of Wright et al. (2010) that showed impairment of rat's behaviour and brain AChE four weeks after DFP exposure.

2.4. Signs of acute toxicosis

Presence or absence of the following signs of acute toxicosis was recorded up to 3 h after poisoning with chlorpyrifos: ataxia, tremors,

tetany, lacrimation, diarrhoea and salivation. Data were expressed as percentage of animals that presented each sign of toxicosis.

2.5. Behavioural analyses

2.5.1. Forced swimming test

The FST was performed in a plastic tank (52 cm height × 24 cm diameter) filled with water (25 cm height) at temperature of 22–24 °C, using an adaptation of Porsolt's method (Porsolt et al., 1977). Briefly, each rat was placed into the cylinder and allowed to swim for 15 min (pre-test session). Twenty-four hours later, the same procedure was repeated for 5 min (test session). In experiment 2, the test session was repeated once again on the 30th day (re-test session). An experimenter blind to treatment groups analysed the videotaped sessions. The immobility time (in seconds) was measured for 5 min in both test and re-test sessions.

2.5.2. Open field test

During open field test sessions, rats were placed in the centre of the apparatus (a 1 m^2 wooden box marked with a grid and square crossings, 30-cm high walls) and allowed to freely explore it for 5 min. The sessions were video-recorded and analysed offline by an experimenter blind to treatment groups. The total number of line crossings (movement of all four paws of the rat across a square and entrance in another) and the number of line crossings in the centre of open field were scored.

2.6. Cholinesterase assays

2.6.1. Plasma butyrylcholinesterase activity

We measured BChE activity as an index of effectiveness of chlorpyrifos treatment. Briefly, trunk blood was collected into 2-ml plastic tubes containing heparin and centrifuged at 4000 rpm during 10 min using a microcentrifuge (Mikro 120, Hettich). Plasma samples were collected and used to measure BChE activity according to a colorimetric method described by Ellman et al. (1961) and modified by Dietz et al. (1973). The enzyme activity was expressed as International Units (I.U./L), being one U of BChE the amount of enzyme that hydrolyses one µmol of propionylthiocholine/min/mL of serum at 37 °C.

2.6.2. Brain acetylcholinesterase activity

Brain AChE activity was measured separately in the prefrontal cortex, hippocampus and striatum using a spectrophotometric method described by Ellman et al. (1961) and modified by Lassiter et al. (2003) and Pires et al. (2005). Briefly, after the brain was quickly removed on ice and brain regions were dissected, aliquots of 20 mg from each area were transferred to 2-ml plastic tubes, and stored at −80 °C until the day of assays. The samples were homogenized in 1 ml of 0.1 M phosphate buffer with 1% Triton X-100, pH 8.0 using a Potter-Elvehjem homogenizer (TE-099, Tecnal, Piracicaba, São Paulo, Brazil) and centrifuged at 7800 G during 5 min at 4 °C (Centrifuge 5804, Eppendorf). For the spectrophotometric assay, 135 µl of the supernatant were added to a cuvette containing 35 µl of 5 mM dithiobisnitrobenzoic acid (DTNB) and 820 µl of 0.1 M phosphate buffer with 1% Triton X-100, pH 8.0. The reaction started by adding 10 µl of 75 mM acetylthiocholine (ATCh). Changes in optical density were monitored at 412 nm (Evolution 300 PC, Thermo Scientific spectrophotometer, Madison, Wisconsin, USA). Protein concentration was determined by Bradford's method (Bradford, 1976). The AChE activity was expressed in micromoles of ATCh hydrolysed per hour per milligram of protein.

2.7. Statistical analysis

All data were screened for outliers using Dixon's test for a single outlier. Data from acute signs of toxicosis were analysed using Generalized Estimating Equations (GEE) through a multinomial ordinal logistic regression. Firstly, we compared saline *vs* chlorpyrifos groups. Then, we analysed if treatment with antidotes affected signs of toxicosis. Immobility time (FST), total number of line crossings and number of line crossings in the central area (Open field) from experiment 1, and plasma BChE and brain AChE activity were analysed by one-way analysis of variance (ANOVA). If there was a main effect in ANOVA, comparisons of all groups *vs* saline, and the 3 antidote + CPF groups *vs* the CFP group, were performed using a two-tailed Dunnett's *post hoc* test. Immobility time (FST) data from experiment 2 were analysed using two-way repeated measures ANOVA. The main factors were treatment and session in the FST (24 h and 30 days after poisoning). If there was a main effect of treatment or interaction between treatment *vs* session, comparisons of all groups *vs* saline were performed using Dunnett's *post hoc* test. We also compared the 3 antidote + CPF groups *vs* CPF group. Statistical significance was set up at $p \leq 0.05$. Data are presented as mean ± SEM.

3. Results

3.1. Experiment 1

A single exposure to chlorpyrifos impaired behaviour in the FST ($F_{3,56} = 3.413$, $p < 0.05$; Fig. 2). Chlorpyrifos 20 mg/kg increased immobility time compared to the control group (Dunnett's *post hoc* test, p = 0,024).

Table 1 shows the effect of three different doses of chlorpyrifos in the open field 24 h after poisoning. None of chlorpyrifos doses changed the number of total line crossings ($F_{3,36} = 1.05$, $p = 0.338$) or the number of line crossings in the central area ($F_{3,36} = 1.01$, $p = 0.404$).

3.2. Experiment 2

3.2.1. Signs of acute toxicosis

After the first hour of poisoning, rats exposed to chlorpyrifos are more likely to present ataxia (Wald-$X^2_{(1)} = 1609.74$; $p < 0.001$), tremors, (Wald-$X^2_{(1)} = 2089.17$; $p < 0.001$), tetany (Wald-$X^2_{(1)} = 7959.91$; $p < 0.000$), lacrimation (Wald-$X^2_{(1)} = 735.87$; $p < 0.001$) and salivation (Wald-$X^2_{(1)} = 537.19$; $p < 0.001$), but not diarrhoea compared to saline group (Table 2). The majority of signs in chlorpyrifos-treated animals had resolved at 2–3 h, which means that there was no significant difference in signs between saline and chlorpyrifos-treated animals at those points. As at 2–3 h there were minimal signs of toxicosis, treatment with the antidotes atropine and/or pralidoxime did not have any effect. None of the rats died during experiments.

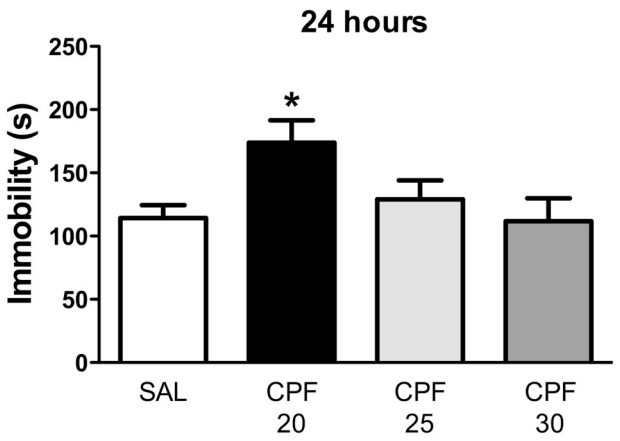

Fig. 2. Effect of a single injection (i.p.) of chlorpyrifos (CPF) 20 mg/kg (n = 15), 25 mg/kg (n = 15), 30 mg/kg (n = 15) or saline (n = 15) on the immobility time (s) of rats subjected to the forced swimming test 24 h after poisoning. Bars represent the mean ± SEM. * $p < 0.05$ compared to saline group (One-way ANOVA followed by Dunnett's *post hoc* test).

Table 1
Locomotor activity in the open field 24 h after a single injection of chlorpyrifos (20, 25 and 30 mg/kg; n = 10) or saline (SAL; n = 10).

Treatment	Total number of line crossings[a]	Number of line crossings in the central area
SAL	95.1 ± 10.06	14.00 ± 2.35
CPF 20 mg/kg	75.7 ± 9.29	8.70 ± 1.63
CPF 25 mg/kg	79.3 ± 7.70	12.10 ± 2.84
CPF 30 mg/kg	74.8 ± 9.58	9.70 ± 2.55

[a] Values represent means ± S.E.M. One-way ANOVA.

3.2.2. Forced swimming test

Fig. 3 shows the effects of chlorpyrifos (20 mg/kg) and post-treatment with atropine and/or pralidoxime on immobility time in the FST 24 h (test session; Fig. 3A) and 30 days (re-test session; Fig. 3B) after acute poisoning. The analyses showed effect of both session ($F_{1,71}$ = 27.93, p < 0.001) and treatment ($F_{7,71}$ = 2.22, p = 0.042), as well as interaction between session and treatment ($F_{7,71}$ = 2.76, p = 0.013). Chlorpyrifos raised immobility time compared to saline group (Dunnett's *post hoc* test, p = 0.001) in the test session (24 h after dosing). Atropine reversed the depressive-like behaviour induced by chlorpyrifos (Dunnett's *post hoc test*, p = 0.050 compared to chlorpyrifos group). Post-treatment with pralidoxime or pralidoxime + atropine did not reverse chlorpyrifos-induced change in immobility time (Dunnett's *post hoc* test, p > 0.05 compared to chlorpyrifos group). Immobility times did not differ in the re-test session 30 days after dosing.

3.2.3. Plasma butyrylcholinesterase activity

For plasma BChE activity, the main effect of treatment was significant 24 h after dosing ($F_{7,31}$ = 13.98, p < 0.001; Fig. 4A). Plasma BChE activity was reduced in all groups treated with chlorpyrifos (CPF, CPF + ATR, CPF + 2-PAM and CPF + ATR + 2-PAM) compared to saline group (Dunnett's *post hoc* test, p < 0.01). Treatment with antidotes did not recover BChE activity in animals poisoned with chlorpyrifos (Dunnett's *post hoc* test, p > 0.05 *vs* chlorpyrifos group). Plasma BChE activity was recovered in all poisoned groups after 30 days of treatment ($F_{7,34}$ = 1.48, p = 0.206; Fig. 4B).

3.2.4. Brain acetylcholinesterase activity

Fig. 5 presents AChE activity in the hippocampus, prefrontal cortex and striatum 24 h and 30 days after poisoning. A single exposure to chlorpyrifos inhibited hippocampal AChE 24 h after treatment ($F_{7,27}$ = 7.40, p < 0.001; Fig. 5A). The enzyme activity in the hippocampus was reduced in all poisoning groups (CPF, CPF + ATR, CPF + 2-PAM and CPF + ATR + 2-PAM) compared to saline group (Dunnett's *post hoc* test, p = 0.013, p = 0.004, p = 0.003 and p = 0.001 respectively). The AChE activity was recovered in the hippocampus 30 days after poisoning ($F_{7,29}$ = 1.84, p = 0.117; Fig. 5D).

Acute chlorpyrifos poisoning decreased the striatal AChE activity 24 h after exposure ($F_{7,29}$ = 3.92, p = 0.004; Fig. 5B). CPF, CPF + ATR and CPF + ATR + 2-PAM groups inhibited AChE compared to saline group (Dunnett's *post hoc* test, p = 0.021, p = 0.028 and p = 0.005 respectively). CPF + 2-PAM did not significantly differ from the saline group (p = 0.065). On the other hand, treatment with pralidoxime did not recover AChE activity 24 h after rats have been poisoned with chlorpyrifos, since CPF + 2-PAM and CPF + ATR + 2-PAM did not differ from CPF group (Dunnett's *post hoc* test, p > 0.05). Thirty days after dosing (Fig. 5E), there was a complete recovery of AChE activity in the striatum ($F_{7,30}$ = 0.69, p = 0.677).

In the prefrontal cortex, the treatment affected AChE activity 24 h after exposure ($F_{7,23}$ = 6.31, p < 0.001; Fig. 5C). CPF, CPF + ATR and CPF + ATR + 2-PAM groups presented significant AChE activity reduction compared to saline group (Dun*nett's post hoc* test, p = 0.001). Treatment with pralidoxime prevented the reduction of AChE activity in the prefrontal cortex of rats poisoned with chlorpyrifos (Dunnett's *post hoc* test, p = 0.028 CPF + 2-PAM *vs* CPF group). No statistical difference was observed in AChE activity in the prefrontal cortex at day 30 ($F_{7,26}$ = 1.91, p = 0.109), as shown in Fig. 5F.

4. Discussion

In the present work, we found that a sub-lethal dose of chlorpyrifos

Table 2
Effects of treatment with saline (SAL), atropine (ATR; 10 mg/kg), pralidoxime (2-PAM, 40 mg/kg) or with combination of atropine and pralidoxime on signs of acute toxicosis induced by chlorpyrifos (CPF, 20 mg/kg).

Treatment	Ataxia[a]	Tremors	Tetany	Lacrimation	Salivation	Diarrhoea
			1 h after poisoning			
SAL (n = 39[b])	0	0	0	0	0	0
CPF (n = 41[c])	22.0*	90.2*	46.3*	17.1*	19.5*	26.8
			2 h after poisoning			
SAL (n = 12)	0	0	0	0	0	0
CPF (n = 13)	7.7	0	0	0	10	0
ATR (n = 9)	0	0	0	0	0	0
CPF + ATR (n = 9)	0	10	10	0	10	0
2-PAM (n = 10)	0	0	0	0	0	0
CPF + 2-PAM (n = 9)	0	0	0	0	0	0
ATR + 2-PAM (n = 8)	0	0	0	0	0	0
CPF + ATR + 2-PAM (n = 9)	0	0	0	0	0	0
			3 h after poisoning			
SAL	0	0	0	0	0	0
CPF	7.7	7.7	0	0	0	7.7
ATR	0	0	0	0	0	0
CPF + ATR	0	10	0	0	0	0
2-PAM	0	0	0	0	0	0
CPF + 2-PAM	0	0	0	0	0	0
ATR + 2-PAM	0	0	0	0	0	0
CPF + ATR + 2-PAM	0	0	0	0	0	0

* p < 0.05 by Generalized Estimated Equation.
[a] Data were expressed as percentage of animals that presented each sign of toxicosis.
[b] This total number of animals was divided, after 1 h, in 4 groups: SAL; ATR; 2-PAM and ATR + 2-PAM.
[c] This total number of animals was divided, after 1 h, in 4 groups: CPF; CPF + ATR; CPF + 2-PAM and CPF + ATR + 2-PAM.

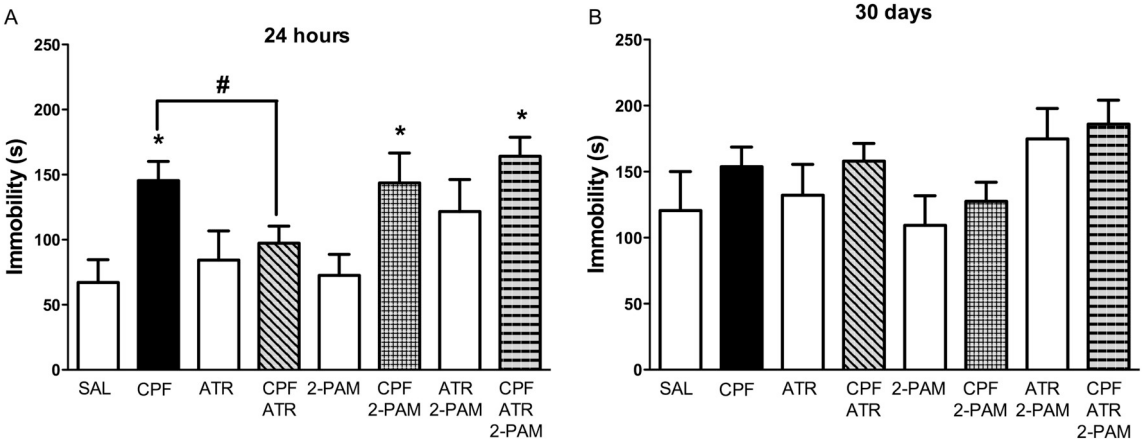

Fig. 3. Effects of a single injection (i.p.) of atropine (ATR, 10 mg/kg) and/or pralidoxime (2-PAM, 40 mg/kg) on the depressive-like behaviour induced by an acute poisoning with chlorpyrifos (CPF, 20 mg/kg) in rats subjected to the forced swimming test 24 h (3A) and 30 days (3B) after poisoning. Experimental groups: SAL (n = 12); CPF (n = 13); ATR (n = 9); CPF + ATR (n = 9); 2-PAM (n = 10); CPF + 2-PAM (n = 9); ATR + 2-PAM (n = 8) and CPF + ATR + 2-PAM (n = 9). Bars represent the mean ± SEM. * $p < 0.05$ compared to saline group. # $p < 0.05$ compared to CPF group (Two-way ANOVA followed by Dunnett's *post hoc* test).

(20 mg/kg) induced depressive-like behaviour in rats submitted to the FST 24 h after dosing. This dose of chlorpyrifos also elicited signs of acute toxicosis within the first hour after poisoning, as well as reduced plasma BChE activity, an useful marker of OP poisoning (Eddleston et al., 2008; Singleton et al., 2015). Altogether, these data confirm that CPF 20 mg/kg induced acute cholinergic toxicity simultaneously an impairment in behaviour. The increase in the immobility time might not be attributed to impairment in spontaneous locomotor activity, since none of chlorpyrifos doses significantly affected the total number of line crossings in the open field. Further, the depressive-like behaviour induced by chlorpyrifos seems to be due to a central action of this OP pesticide, considering that AChE activity in the hippocampus, striatum and prefrontal cortex was reduced 24 h after treatment. A single injection of atropine attenuated the depressive-like behaviour induced by chlorpyrifos in the FST 24 h after poisoning whereas a single injection of pralidoxime did not prevent it. In the same vein, pralidoxime showed no effect in the inhibition of hippocampal and striatal AChE activity noticed 24 h after poisoning. In contrast, pralidoxime partially reactivated AChE activity in the prefrontal cortex. The combination of atropine and pralidoxime had no effect on either behavioural and biochemical changes elicited by chlorpyrifos. Also, the behavioural and biochemical consequences of a single sub-lethal exposure to chlorpyrifos were non-persistent, as rats re-tested in the FST showed no changes on immobility time and they recovered plasma BChE and brain AChE activity 30 days after poisoning.

It is important to note that we adopted a commercial formulation of chlorpyrifos instead of pure compound to induce intoxication and to evaluate effectiveness of standard antidotes. This experimental design provides similar conditions to the clinical settings and guarantees the translational aspect of the study. Acute cholinergic signs and decreased AChE activity may ascertain the effectiveness of intoxication by chlorpyrifos. At the same time, we cannot exclude the influence of other ingredients presented in the commercial formulation. Beyond the active ingredient chlorpyrifos, the main ingredient of the commercial formulation of chlorpyrifos is heavy aromatic naphtha (52% m/v, Lorsban). Exposure to concentrations of heavy aromatic naphtha at levels much higher than used in our study seemed to induce only mild CNS effects (Amoruso et al., 2008). Additionally, there are no studies showing that heavy aromatic naphtha inhibits brain AChE. Nevertheless, further studies administering pure chlorpyrifos systemically or directly in the brain are necessary to elucidate the mechanism of action underlying its depressive-like effect. Regarding the administration of

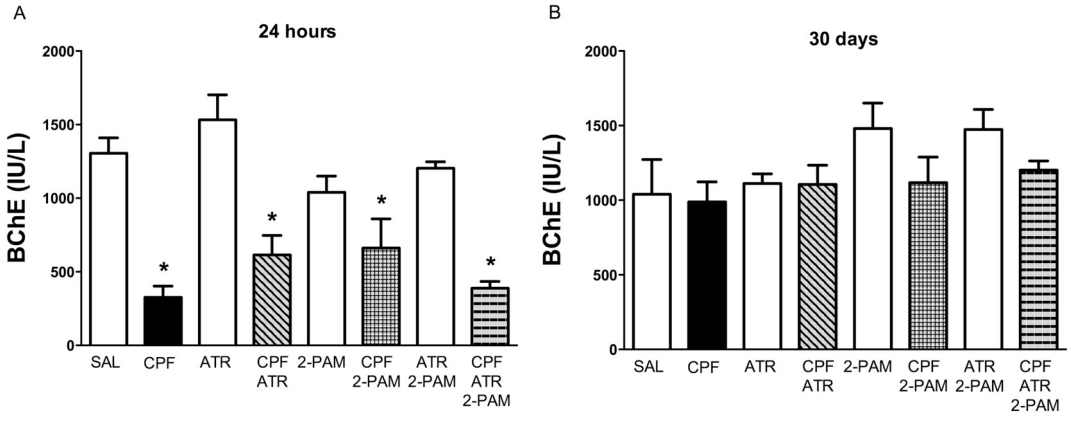

Fig. 4. Effect of a single injection of chlorpyrifos (CPF; 20 mg/kg) and post-treatment with atropine (ATR; 10 mg/kg) and/or pralidoxime (2-PAM; 40 mg/kg) on plasma butyrylcholinesterase (BChE) activity 24 h (4A) and 30 days (4B) after poisoning. 4A) Rats were injected (i.p.) with saline (SAL) or CPF and after 1 h treated with ATR, 2-PAM or the combination of ATR plus 2-PAM to measure BChE 24 h post-poisoning: SAL (n = 5); CPF (n = 6); ATR (n = 4); CPF + ATR (n = 6); 2-PAM (n = 5); CPF + 2-PAM (n = 5); ATR + 2-PAM (n = 5) and CPF + ATR + 2-PAM (n = 5). 4B) Rats were injected (i.p.) with saline (SAL) or CPF and after 1 h treated with ATR, 2-PAM or the combination of ATR plus 2-PAM to measure BChE 30 days post-poisoning: SAL (n = 6); CPF (n = 5); ATR (n = 4); CPF + ATR (n = 5); 2-PAM (n = 7); CPF + 2-PAM (n = 5); ATR + 2-PAM (n = 5) and CPF + ATR + 2-PAM (n = 5). Bars represent the mean ± SEM. * $p < 0.05$ compared to saline group. (One-way ANOVA followed by Dunnett's *post hoc* test).

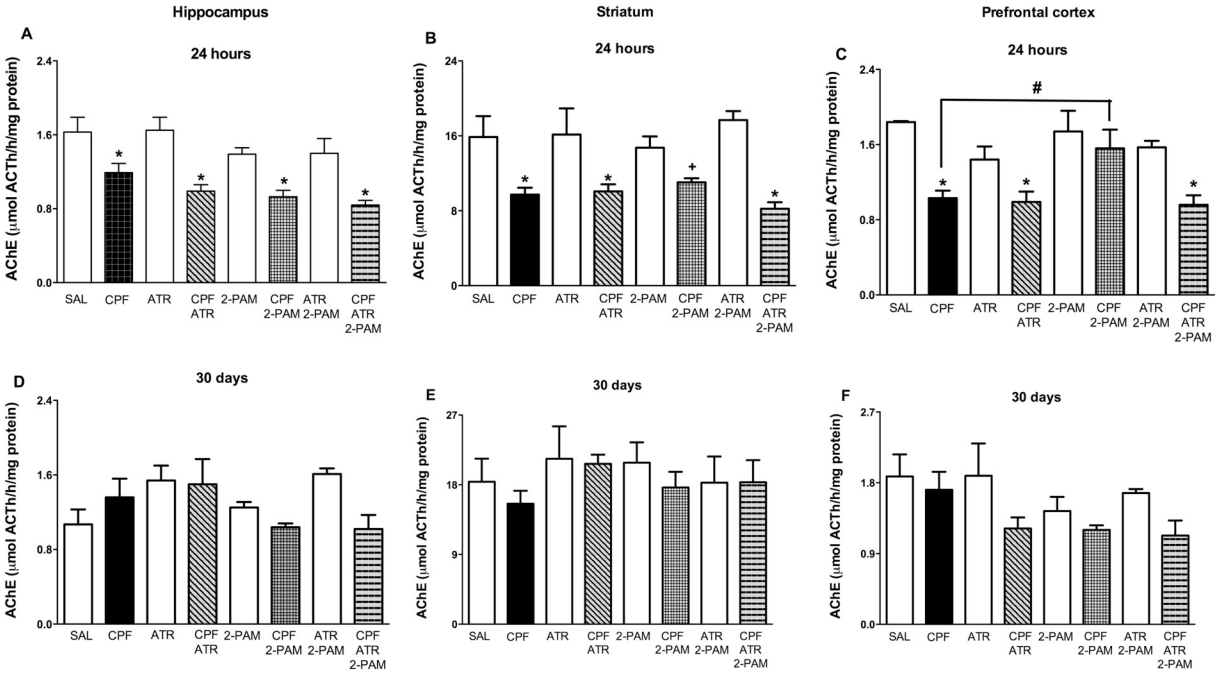

Fig. 5. Effect of a single injection (i.p.) of chlorpyrifos (CPF; 20 mg/kg) and post-treatment (1 h after) with atropine (ATR; 10 mg/kg) and/or pralidoxime (2-PAM; 40 mg/kg) on acetylcholinesterase (AChE) activity in the hippocampus (5A and 5D), striatum (5B and 5E) and prefrontal cortex (5C and 5F) 24 h and 30 days after poisoning. Group sizes vary from 4 to 8. Bars represent the mean ± SEM * $p < 0.05$ compared to saline group. + $p = 0.065$ compared to saline group; # $p < 0.05$ compared to CPF group (One-way ANOVA followed by Dunnett's *post hoc* test).

chlorpyrifos by the i.p. route, it is important to recognize the loss of the translational aspect but the achievement of a more controlled intoxication profile.

Our results showed that the lowest, but not the highest doses of chlorpyrifos, induced depressive-like behaviour at the same time that it reduced brain AChE activity. This parallels the findings of other studies in which low but not high doses of OP pesticides induced depressive-like behaviour (Chen et al., 2014, 2011; Lima et al., 2009). Besides that, acute or repeatedly exposure to other OP pesticide, malathion, also prompted depressive-like behaviour associated with brain AChE inhibition (Assini et al., 2005; Ramos et al., 2006; Saeedi Saravi et al., 2016). Hence, we speculate that chlorpyrifos, by inhibiting brain AChE, raised cholinergic neurotransmission in brain areas involved with depression, such as the hippocampus. Direct overstimulation of brain cholinergic receptors or indirect changes in central neurotransmission might explain the depressive-like behaviour induced by an acute and sub-lethal exposure to chlorpyrifos. Our hypothesis is supported by some studies showing that different schedules of organophosphates treatment change serotonergic, glutamatergic and gabaergic neurotransmission (Gubert et al., 2011; Judge et al., 2016; Moreno et al., 2008; Shih and McDonough, 1997). Thus, considering that, in the present study, only the lowest dose of chlorpyrifos (20 mg/kg) induced a depressive-like behaviour, we speculate that this dose had affected serotonergic or glutamatergic neurotransmission in the hippocampus. On the other hand, it is possible that the higher doses of chlorpyrifos did not affect the same neurotransmitters or even affected different neurotransmitters, which would explain the absence of depressive-like effect by these doses. Further studies will be necessary to test this hypothesis.

Janowsky et al. (1972) postulated that an imbalance between central adrenergic and cholinergic neurotransmission could precipitate affective disorders, such as depression and mania. They proposed a predominance of the cholinergic system in depression and a predominance of the adrenergic system in mania (Janowsky et al., 1972). A plenty body of evidence support the involvement of cholinergic neurotransmission in depression. For instance, physostigmine (a central AChE inhibitor), but not neostigmine (a peripheral cholinesterase inhibitor), increased symptoms that resembling depression (Janowsky et al., 1986), suggesting that a brain excess of ACh is involved in this behavioural impairment. In the same way, only a centrally acting muscarinic antagonist blocked the behavioural effects of physostigmine (Janowsky et al., 1986). In addition, physostigmine injected directly in mice hippocampus also induced a depressive-like behaviour (Mineur et al., 2013). Muscarinic antagonists also exhibited antidepressant effect in pre-clinical and clinical studies (Drevets et al., 2013; Mancinelli et al., 1988; Witkin et al., 2014). Thus, it is possible that chlorpyrifos elicited a depressive-like effect possibly by facilitating the activation of muscarinic receptors in areas such as the hippocampus and the prefrontal cortex. Corroborating our hypothesis, atropine, a muscarinic antagonist, counteracted the depressive-like effect induced by a single administration of chlorpyrifos.

Regarding treatment with the antidote pralidoxime, a single administration showed no effect on chlorpyrifos-induced depressive-like behaviour in the FST and did not reactivate hippocampal and striatal AChE, but partially recovered cortical AChE activity. At this point, there is no consensus about the oximes's effectiveness to reactivate the central AChE (Bajgar et al., 2007; Voicu et al., 2010b). For example, HI-6, another oxime, partially reactivated AChE in the prefrontal cortex, but did not in the hippocampus (Clement, 1992). On the other hand, several oximes did not prevent the inhibition of AChE induced by OP exposure in most brain regions, at least 1 h after poisoning (Bajgar et al., 2012; Shih et al., 2011, 2009). Therefore, although it is merely speculative, it is possible that pralidoxime did not block the depressive-like behaviour of animals poisoned by chlorpyrifos due to its inability to reactivate hippocampal or striatal AChE.

Non-cholinergic mechanisms could also be related to the behavioural effects induced by chlorpyrifos (Judge et al., 2016), which has been seen in studies involving repeated or developmental exposures (Aldridge et al., 2005, 2003; Moreno et al., 2008; Naughton and Terry, 2018; Terry, 2012). Indeed, OP poisoning can trigger overstimulation of muscarinic receptors, excitotoxicity, oxidative stress and neuroinflammation (Chen, 2012; López-Granero et al., 2013), changing many

enzymatic functions (Abdel-Rahman et al., 2002; Terry, 2012). In fact, there is a body of evidence showing that even acute OP exposure results in neuroinflammation and other neurotoxic effects in brain areas such as hippocampus during the first 24 h after dosing (Banks and Lein, 2012; Naughton and Terry, 2018). In the same vein, acute exposure to soman, another OP compound, inhibited brain AChE (Shih and McDonough, 1997) and increased cytokines expression in the hippocampus during the first 24 h (Johnson and Kan, 2010). Scopolamine, another muscarinic receptor antagonist, was neuroprotective against glutamate excitotoxicity in hippocampal cells culture of rats (Rami et al., 1997) and also was effective against soman-elicited neurotoxic effects (McDonough et al., 1989). Given that, it is plausible that chlorpyrifos, by inhibiting hippocampal AChE, generated neuroinflammation that, indirectly, succeed in a depressive-like effect. Conversely, atropine, a muscarinic antagonist, could have mitigated the neurotoxicity induced by chlorpyrifos to attenuate the behavioural impairment.

In our study, the combination of atropine and pralidoxime did not prevent the impairment of behavioural and biochemical parameters prompted by chlorpyrifos, despite some evidence indicating that this treatment presents a synergistic effect in the OP poisoning management (Askew, 1957; Lundy and Shih, 1983). Supporting our findings, Svensson et al. (2005) also showed that atropine is more effective than the combination of atropine and HI-6 (an oxime) to prevent simultaneously seizures and up-regulation of a pro-inflammatory cytokine by soman poisoning. Furthermore, the association of atropine and pralidoxime did not exhibit considerable advantage over atropine-only treatment in patients exposed to OP pesticides (Banerjee et al., 2014; Blumenberg et al., 2018). One possible explanation to these unexpected results is that atropine could preserve the integrity of the blood-brain barrier, restricting the access of oximes through the CNS when these antidotes were given simultaneously. In fact, pralidoxime is a quaternary monopyridinum compound with poor blood-brain barrier penetration (Sakurada et al., 2003; Voicu et al., 2010a).

Although in the present study rats exhibited signs of acute toxicosis in the first hour after chlorpyrifos injection, the majority of these signs were lost after 2–3 h. Thus, considering that there were minimal chlorpyrifos related-signs of toxicosis, we were not able to show any effect of treatment with atropine plus pralidoxime in the test of acute toxicosis.

Surprisingly, 30 days after chlorpyrifos poisoning, activity of plasma BChE and brain AChE, as well as the immobility time in the FST were recovered, suggesting that the consequences of acute exposure to a sublethal dose of chlorpyrifos are transient. In contrast, some authors reported persistent cognitive impairment and brain AChE activity inhibition after acute exposure to higher doses of chlorpyrifos (Bushnell et al., 1993; López-Crespo et al., 2007; López-Granero et al., 2013; Pope et al., 1992) or DFP (Wright et al., 2010). On possible explanation to these different results refers to the tested doses. Brown and Brix (1998) proposed that the outcomes of acute OP poisoning are closely related to the level of exposure and initial signs and symptoms observed. According to them, high-level exposure causing definitive cholinergic signs and symptoms predict long-term consequences. Considering that in our study, a single exposure to chlorpyrifos induced cholinergic signs and symptoms in no > 50% of the animals, it maybe that the level of exposure was not been high enough to induce long-term impairment. Beyond employed doses, significant differences between these aforementioned studies and ours involve the route of administration of the OP compound and the vehicle of administration. Those studies administered the OP compound by subcutaneous route while we administered by i.p. route. Moreover, we used a saline solution to prepare chlorpyrifos and the other studies used oil. Results from experimental and simulation data suggest that different routes and vehicles of chlorpyrifos administration could lead to different body burdens of the OP compound, different rates of bioactivation to chlorpyrifos-oxon and, consequently, to different toxic responses (Smith et al., 2009). After all, these procedural differences might explain why we did not notice inhibition of cholinesterase 30 days after acute poisoning with chlorpyrifos compared to the other studies mentioned above.

5. Conclusions

To sum up, our results indicated that acute exposure to a commercial formulation of chlorpyrifos elicited transient behavioural and biochemical changes that were partially reversed by the antidotes atropine and pralidoxime. Moreover, the depressive-like behaviour induced by a single injection of chlorpyrifos might be due to inhibition of striatal or hippocampal AChE, since pralidoxime did not reactivate the enzyme in this brain region as well as did not reverse the behavioural impairment. Finally, our study suggests that even acute OP poisoning may cause short-term impairment to the mental health of subjects exposed to these compounds and that a single dose of the standard treatment of OP poisoning seems to be insufficient to reverse all OP-induced impairments.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ntt.2018.11.002.

Transparency document

The Transparency document associated with this article can be found, in online version.

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References

Abdel-Rahman, A., Shetty, A.K., Abou-Donia, M.B., 2002. Acute exposure to sarin increases blood brain barrier permeability and induces neuropathological changes in the rat brain: dose-response relationships. Neuroscience 113, 721–741. https://doi.org/10.1016/S0306-4522(02)00176-8.

Aldridge, J.E., Seidler, F.J., Meyer, A., Thillai, I., Slotkin, T.A., 2003. Serotonergic systems targeted by developmental exposure to chlorpyrifos: effects during different critical periods. Environ. Health Perspect. 111, 1736–1743. https://doi.org/10.1289/ehp.6489.

Aldridge, J.E., Levin, E.D., Seidler, F.J., Slotkin, T.A., 2005. Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. Environ. Health Perspect. 113, 527–531. https://doi.org/10.1289/ehp.7867.

Amoruso, M.A., Gamble, J.F., McKee, R.H., Rohde, A.M., Jaques, A., 2008. Review of the toxicology of mineral spirits. Int. J. Toxicol. 27, 97–165. https://doi.org/10.1080/10915810701876786.

Askew, B.M., 1957. Oximes and atropine in sarin poisoning. Br. J. Pharmacol. Chemother. 12, 340–343.

Assini, F.L., Zanette, K.D., Brocardo, P.S., Pandolfo, P., Rodrigues, A.L.S., Takahashi, R.N., 2005. Behavioral effects and ChE measures after acute and repeated administration of malathion in rats. Environ. Toxicol. Pharmacol. 20, 443–449. https://doi.org/10.1016/j.etap.2005.05.007.

Bajgar, J., Kuca, K., Jun, D., Bartosova, L., Fusek, J., 2007. Cholinesterase reactivators: the fate and effects in the organism poisoned with organophosphates/nerve agents. Curr. Drug Metab. 8, 803–809. https://doi.org/10.2174/138920007782798144.

Bajgar, J., Hajek, P., Kassa, J., Slizova, D., Krs, O., Karasova, J.Z., Fusek, J., Capek, L., Voicu, V.A., 2012. Combined approach to demonstrate acetylcholinesterase activity changes in the rat brain following tabun intoxication and its treatment. Toxicol. Mech. Methods 22, 60–66. https://doi.org/10.3109/15376516.2011.596231.

Banerjee, I., Tripathi, S., Roy, A.S., 2014. Efficacy of pralidoxime in organophosphorus poisoning: revisiting the controversy in Indian setting. J. Postgrad. Med. 60, 27–30. https://doi.org/10.4103/0022-3859.128803.

Banks, C.N., Lein, P.J., 2012. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology 33, 575–584.

https://doi.org/10.1016/j.neuro.2012.02.002.
Beseler, C., Stallones, L., Hoppin, J.A., Alavanja, M.C.R., Blair, A., Keefe, T., Kamel, F., 2006. Depression and pesticide exposures in female spouses of licensed pesticide applicators in the agricultural health study cohort. J. Occup. Environ. Med. 48, 1005–1013. https://doi.org/10.1097/01.jom.0000235938.70212.dd.
Blumenberg, A., Benabbas, R., Desouza, I.S., Conigliaro, A., Paladino, L., Warman, E., Sinert, R., Wiener, S.W., 2018. Utility of 2-pyridine aldoxime methyl chloride (2-PAM) for acute organophosphate poisoning: a systematic review and meta-analysis. J. Med. Toxicol. https://doi.org/10.1007/s13181-017-0636-2.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Braquenier, J.B., Quertemont, E., Tirelli, E., Plumier, J.C., 2010. Anxiety in adult female mice following perinatal exposure to chlorpyrifos. Neurotoxicol. Teratol. 32, 234–239. https://doi.org/10.1016/j.ntt.2009.08.008.
Brown, M.A., Brix, K.A., 1998. Review of health consequences from high-, intermediate- and low level exposure to organophosphorus nerve agents. J. Appl. Toxicol. 18, 393–408.
Buckley, N.A., Eddleston, M., Li, Y., Bevan, M., Robertson, J., 2011. Oximes for acute organophosphate pesticide poisoning. Cochrane Database Syst. Rev., CD005085. https://doi.org/10.1002/14651858.CD005085.pub2.
Bushnell, P.J., Pope, C.N., Padilla, S., 1993. Behavioral and neurochemical effects of acute chlorpyrifos in rats: tolerance to prolonged inhibition of cholinesterase. J. Pharmacol. Exp. Ther. 266, 1007–1017.
Chen, Y., 2012. Organophosphate-induced brain damage: mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. Neurotoxicology 33, 391–400. https://doi.org/10.1016/j.neuro.2012.03.011.
Chen, W.Q., Yuan, L., Xue, R., Li, Y.F., Su, R.B., Zhang, Y.Z., Li, J., 2011. Repeated exposure to chlorpyrifos alters the performance of adolescent male rats in animal models of depression and anxiety. Neurotoxicology 32, 355–361. https://doi.org/10.1016/j.neuro.2011.03.008.
Chen, W.Q., Zhang, Y.Z., Yuan, L., Li, Y.F., Li, J., 2014. Neurobehavioral evaluation of adolescent male rats following repeated exposure to chlorpyrifos. Neurosci. Lett. 570, 76–80. https://doi.org/10.1016/j.neulet.2014.03.068.
Clement, J.G., 1992. Central activity of acetylcholinesterase oxime reactivators. Toxicol. Appl. Pharmacol. 112, 104–109. https://doi.org/10.1016/0041-008X(92)90285-Z.
Colosio, C., Tiramani, M., Maroni, M., 2003. Neurobehavioral effects of pesticides: state of the art. Neurotoxicology 24, 577–591. https://doi.org/10.1016/S0161-813X(03)00055-X.
Cunha, A.F., Felippe, I.S.A., Ferreira-Junior, N.C., Resstel, L.B.M., Guimarães, D.A.M., Beijamini, V., Paton, J.F.R., Sampaio, K.N., 2018. Neuroreflex control of cardiovascular function is impaired after acute poisoning with chlorpyrifos, an organophosphorus insecticide: possible short and long term clinical implications. Toxicology 398–399, 13–22. https://doi.org/10.1016/j.tox.2018.02.005.
de Silva, H.J., Wijewickrema, R., Senanayake, N., 1992. Does pralidoxime affect outcome of management in acute organophosphorus poisoning? Lancet 339, 1136–1138. https://doi.org/10.1016/0140-6736(92)90733-J.
Dietz, A.A., Rubinstein, H.M., Lubrano, T., 1973. Colorimetric determination of serum cholinesterase and its genetic variants by the propionylthiocholine-dithiobis(nitrobenzoic acid)procedure. Clin. Chem. 19, 1309–1313.
Drevets, W.C., Zarate, C.A., Furey, M.L., 2013. Antidepressant effects of the muscarinic cholinergic receptor antagonist scopolamine: a review. Biol. Psychiatry 73, 1156–1163. https://doi.org/10.1016/j.biopsych.2012.09.031.
Eddleston, M., 2018. Are oximes still indicated for acute organophosphorus insecticide self-poisoning? J. Med. Toxicol. 1–2. https://doi.org/10.1007/s13181-018-0651-y.
Eddleston, M., Buckley, N.A., Eyer, P., Dawson, A.H., 2008. Management of acute organophosphorus pesticide poisoning. Lancet 371, 597–607. https://doi.org/10.1016/S0140-6736(07)61202-1.
Eddleston, M., Eyer, P., Worek, F., Juszczak, E., Alder, N., Mohamed, F., Senarathna, L., Hittarage, A., Azher, S., Jeganathan, K., Jayamanne, S., Von Meyer, L., Dawson, A.H., Sheriff, M.H.R., Buckley, N.A., 2009. Pralidoxime in acute organophosphorus insecticide poisoning - a randomised controlled trial. PLoS Med. 6. https://doi.org/10.1371/journal.pmed.1000104.
Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric of acetylcholinesterase determination. Biochem. Pharmacol. 7, 88–95.
Gubert, P., Ávila, D.S., Bridi, J.C., Saurin, S., Lugokenski, T.H., Villarinho, J.G., Fachinetto, R., Pereira, M.E., Ferreira, J., Batista, J.B.T., Soares, A., 2011. Low concentrations of methamidophos do not alter AChE activity but modulate neurotransmitters uptake in hippocampus and striatum in vitro. Life Sci. 88, 89–95. https://doi.org/10.1016/j.lfs.2010.10.031.
Gunnell, D., Fernando, R., Hewagama, M., Priyangika, W.D.D., Konradsen, F., Eddleston, M., 2007. The impact of pesticide regulations on suicide in Sri Lanka. Int. J. Epidemiol. 36, 1235–1242. https://doi.org/10.1093/ije/dym164.
Jaga, K., Dharmani, C., 2007. The interrelation between organophosphate toxicity and the epidemiology of depression and suicide. Rev. Environ. Health 22, 57–73.
Janowsky, D.S., El-Yousef, M.K., Davis, J.M., Sekerke, H.J., 1972. A cholinergic-adrenergic hypothesis of mania and depression. Lancet 2, 632–635.
Janowsky, D.S., Risch, S.C., Kennedy, B., Ziegler, M., Huey, L., 1986. Central muscarinic effects of physostigmine on mood, cardiovascular function, pituitary and adrenal neuroendocrine release. Psychopharmacology 89, 150–154. https://doi.org/10.1007/BF00310619.
Johnson, E.A., Kan, R.K., 2010. The acute phase response and soman-induced status epilepticus: temporal, regional and cellular changes in rat brain cytokine concentrations. J. Neuroinflammation 7, 1–9. https://doi.org/10.1186/1742-2094-7-40.
Jokanovic, M., 2009. Medical treatment of acute poisoning with organophosphorus and carbamate pesticides. Toxicol. Lett. 190, 107–115. https://doi.org/10.1016/j.toxlet.2009.07.025.
Jokanović, M., Kosanović, M., 2010. Neurotoxic effects in patients poisoned with organophosphorus pesticides. Environ. Toxicol. Pharmacol. 29, 195–201. https://doi.org/10.1016/j.etap.2010.01.006.
Judge, S.J., Savy, C.Y., Campbell, M., Dodds, R., Gomes, L.K., Laws, G., Watson, A., Blain, P.G., Morris, C.M., Gartside, S.E., 2016. Mechanism for the acute effects of organophosphate pesticides on the adult 5-HT system. Chem. Biol. Interact. 245, 82–89. https://doi.org/10.1016/j.cbi.2015.12.014.
Kose, A., Gunay, N., Yildirim, C., Tarakcioglu, M., Sari, I., Demiryurek, A.T., 2009. Cardiac damage in acute organophosphate poisoning in rats: effects of atropine and pralidoxime. Am. J. Emerg. Med. 27, 169–175. https://doi.org/10.1016/j.ajem.2008.01.016.
Kumar, Mr, Vignan Kumar, G., Kumar, Ss., Subrahmanyam, B., Veeraprasad, M., Rammohan, P., Srinivas, M., Babu, Pr, Agrawal, A., 2014. A retrospective analysis of acute organophosphorus poisoning cases admitted to the tertiary care teaching hospital in South India. Ann. Afr. Med. 13, 71. https://doi.org/10.4103/1596-3519.129876.
Lassiter, T.L., Marshall, R.S., Jackson, L.C., Hunter, D.L., Vu, J.T., Padilla, S., 2003. Automated measurement of acetylcholinesterase activity in rat peripheral tissues. Toxicology 186, 241–253. https://doi.org/10.1016/S0300-483X(02)00752-7.
Lekei, E., Ngowi, A.V., London, L., 2014. Hospital-based surveillance for acute pesticide poisoning caused by neurotoxic and other pesticides in Tanzania. Neurotoxicology 45, 318–326. https://doi.org/10.1016/j.neuro.2014.02.007.
Lima, C.S., Ribeiro-Carvalho, A., Filgueiras, C.C., Manhães, A.C., Meyer, A., Abreu-Villaça, Y., 2009. Exposure to methamidophos at adulthood elicits depressive-like behavior in mice. Neurotoxicology 30, 471–478. https://doi.org/10.1016/j.neuro.2009.01.009.
London, L., Flisher, A.J., Wesseling, C., Mergler, D., Kromhout, H., 2005. Suicide and exposure to organophosphate insecticides: cause or effect? Am. J. Ind. Med. 47, 308–321. https://doi.org/10.1002/ajim.20147.
López-Crespo, G. a, Carvajal, F., Flores, P., Sánchez-Santed, F., Sánchez-Amate, M.C., 2007. Time course of biochemical and behavioural effects of a single high dose of chlorpyrifos. Neurotoxicology 28, 541–547. https://doi.org/10.1016/j.neuro.2007.01.013.
López-Crespo, G.A., Flores, P., Sánchez-Santed, F., Sánchez-Amate, M.C., 2009. Acute high dose of chlorpyrifos alters performance of rats in the elevated plus-maze and the elevated T-maze. Neurotoxicology 30, 1025–1029. https://doi.org/10.1016/j.neuro.2009.07.009.
López-Granero, C., Cañadas, F., Cardona, D., Yu, Y., Giménez, E., Lozano, R., Avila, D.S., Aschner, M., Sánchez-Santed, F., 2013. Chlorpyrifos-, diisopropylphosphorofluoridate-, and parathion-induced behavioral and oxidative stress effects: are they mediated by analogous mechanisms of action? Toxicol. Sci. 131, 206–216. https://doi.org/10.1093/toxsci/kfs280.
Lundy, P.M., Shih, T.M., 1983. Examination of the role of central cholinergic mechanisms in the therapeutic effects of HI-6 in organophosphate poisoning. J. Neurochem. 40, 1321–1328.
Lyu, C.P., Pei, J.R., Beseler, L.C., Li, Y.L., Li, J.H., Ren, M., Stallones, L., Ren, S.P., 2018. Case control study of impulsivity, aggression, pesticide exposure and suicide attempts using pesticides among farmers. Biomed. Environ. Sci. 31, 242–246. https://doi.org/10.3967/bes2018.031.
Mancinelli, A., Borsini, F., D'Aranno, V., Lecci, A., Meli, A., 1988. Cholinergic drug effects on antidepressant-induced behaviour in the forced swimming test. Eur. J. Pharmacol. 158, 199–205. https://doi.org/10.1016/0014-2999(88)90067-2.
McDonough, J.H., Jaax, N.K., Crowley, R.A., Mays, M.Z., Modrow, H.E., 1989. Atropine and/or diazepam therapy protects against soman-induced neural and cardiac pathology. Fundam. Appl. Toxicol. 13, 256–276.
Mezadri, T.J., Batista, G.M., Portes, A.C., Marino-Neto, J., Lino-de-Oliveira, C., 2011. Repeated rat-forced swim test: reducing the number of animals to evaluate gradual effects of antidepressants. J. Neurosci. Methods 195, 200–205. https://doi.org/10.1016/j.jneumeth.2010.12.015.
Mileson, B.E., Chambers, J.E., Chen, W.L., Dettbarn, W., Ehrich, M., Eldefrawi, A.T., Gaylor, D.W., Hamernik, K., Hodgson, E., Karczmar, A.G., Padilla, S., Pope, C.N., Richardson, R.J., Saunders, D.R., Sheets, L.P., Sultatos, L.G., Wallace, K.B., 1998. Common mechanism of toxicity: a case study of organophosphorus pesticides. Toxicol. Sci. 41, 8–20. https://doi.org/10.1006/TOXS.1997.2431.
Mineur, Y.S., Obayemi, A., Wigestrand, M.B., Fote, G.M., Calarco, C.A., Li, A.M., Picciotto, M.R., 2013. Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. Proc. Natl. Acad. Sci. U. S. A. 110, 3573–3578. https://doi.org/10.1073/pnas.1219731110.
Moreno, M., Cañadas, F., Cardona, D., Suñol, C., Campa, L., Sánchez-Amate, M.C., Flores, P., Sánchez-Santed, F., 2008. Long-term monoamine changes in the striatum and nucleus accumbens after acute chlorpyrifos exposure. Toxicol. Lett. 176, 162–167. https://doi.org/10.1016/j.toxlet.2007.11.003.
Naughton, S.X., Terry, A.V., 2018. Neurotoxicity in acute and repeated organophosphate exposure. Toxicology 408, 101–112. https://doi.org/10.1016/j.tox.2018.08.011.
Nostrandt, A.C., Padilla, S., Moser, V.C., 1997. The relationship of oral chlorpyrifos effects on behavior, cholinesterase inhibition, and muscarinic receptor density in rat. Pharmacol. Biochem. Behav. 58, 15–23.
Pawar, K.S., Bhoite, R.R., Pillay, C.P., Chavan, S.C., Malshikare, D.S., Garad, S.G., 2006. Continuous pralidoxime infusion versus repeated bolus injection to treat organophosphorus pesticide poisoning: a randomised controlled trial. Lancet 368, 2136–2141. https://doi.org/10.1016/S0140-6736(06)69862-0.
Pires, R.G.W., Pereira, S.R.C., Oliveira-Silva, I.F., Franco, G.C., Ribeiro, A.M., 2005. Cholinergic parameters and the retrieval of learned and re-learned spatial information: a study using a model of Wernicke-Korsakoff Syndrome. Behav. Brain Res. 162, 11–21. https://doi.org/10.1016/j.bbr.2005.02.032.

Pope, C.N., Chakraborti, T.K., Chapman, M.L., Farrar, J.D., 1992. Long-term neurochemical and behavioral effects induced by acute chlorpyrifos treatment. Pharmacol. Biochem. Behav. 42, 251–256. https://doi.org/10.1016/0091-3057(92)90523-I.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732. https://doi.org/10.1038/266730a0.

Rami, A., Ausmeir, F., Winckler, J., Krieglstein, J., 1997. Differential effects of scopolamine on neuronal survival in ischemia and glutamate neurotoxicity: relationships to the excessive vulnerability of the dorsoseptal hippocampus. J. Chem. Neuroanat. 13, 201–208. https://doi.org/10.1016/S0891-0618(97)00044-6.

Ramos, Z.R., Fortunato, J.J., Agostinho, F.R., Martins, M.R., Correa, M., Schetinger, M.R., Dal-Pizzol, F., Quevedo, J., 2006. Influence of malathion on acetylcholinesterase activity in rats submitted to a forced swimming test. Neurotox. Res. 9, 285–290.

Recena, M.C.P., Caldas, E.D., Pires, D.X., Pontes, E.R.J.C., 2006a. Pesticides exposure in Culturama, Brazil—knowledge, attitudes, and practices. Environ. Res. 102, 230–236. https://doi.org/10.1016/j.envres.2006.01.007.

Recena, M.C.P., Pires, D.X., Caldas, E.D., 2006b. Acute poisoning with pesticides in the state of Mato Grosso do Sul, Brazil. Sci. Total Environ. 357, 88–95. https://doi.org/10.1016/j.scitotenv.2005.04.029.

Rosenstock, L., Keifer, M., Daniell, W.E., McConnell, R., Claypoole, K., 1991. Chronic central nervous system effects of acute organophosphate pesticide intoxication. The Pesticide Health Effects Study Group. Lancet 338, 223–227.

Saeedi Saravi, S.S., Amirkhanloo, R., Arefidoust, A., Yaftian, R., Saeedi Saravi, S.S., Shokrzadeh, M., Dehpour, A.R., 2016. On the effect of minocycline on the depressive-like behavior of mice repeatedly exposed to malathion: interaction between nitric oxide and cholinergic system. Metab. Brain Dis. 31, 549–561. https://doi.org/10.1007/s11011-015-9764-z.

Sakurada, K., Matsubara, K., Shimizu, K., Shiono, H., Seto, Y., Tsuge, K., Yoshino, M., Sakai, I., Mukoyama, H., Takatori, T., 2003. Pralidoxime iodide (2-PAM) penetrates across the blood-brain barrier. Neurochem. Res. 28, 1401–1407. https://doi.org/10.1023/A:1024960819430.

Sánchez-Amate, M.C., Flores, P., Sánchez-Santed, F., 2001. Effects of chlorpyrifos in the plus-maze model of anxiety. Behav. Pharmacol. 12, 285–292. https://doi.org/10.1097/00008877-200107000-00007.

Savage, E.P., Keefe, T.J., Mounce, L.M., Heaton, R.K., Lewis, J.A., Burcar, P.J., 1988. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch. Environ. Health. https://doi.org/10.1080/00039896.1988.9934372. An Int. J.

Shih, T.M., McDonough, J.H., 1997. Neurochemical mechanisms in soman-induced seizures. J. Appl. Toxicol. 17, 255–264. https://doi.org/10.1002/(SICI)1099-1263(199707)17:4<255::AID-JAT441>3.0.CO;2-D.

Shih, T.M., Skovira, J.W., O'Donnell, J.C., McDonough, J.H., 2009. In vivo reactivation by oximes of inhibited blood, brain and peripheral tissue cholinesterase activity following exposure to nerve agents in guinea pigs. Chem. Biol. Interact. 187, 207–214. https://doi.org/10.1016/j.cbi.2010.03.006.

Shih, T.M., Guarisco, J.A., Myers, T.M., Kan, R.K., McDonough, J.H., 2011. The oxime pro-2-PAM provides minimal protection against the CNS effects of the nerve agents sarin, cyclosarin, and VX in guinea pigs. Toxicol. Mech. Methods 21, 53–62. https://doi.org/10.3109/15376516.2010.529190.

Silva, J.G., Boareto, A.C., Schreiber, A.K., Redivo, D.D.B., Gambeta, E., Vergara, F., Morais, H., Zanoveli, J.M., Dalsenter, P.R., 2017. Chlorpyrifos induces anxiety-like behavior in offspring rats exposed during pregnancy. Neurosci. Lett. 641, 94–100. https://doi.org/10.1016/j.neulet.2017.01.053.

Singleton, S.T., Lein, P.J., Dadson, O.A., McGarrigle, B.P., Farahat, F.M., Farahat, T., Bonner, M.R., Fenske, R.A., Galvin, K., Lasarev, M.R., Anger, W.K., Rohlman, D.S., Olson, J.R., 2015. Longitudinal assessment of occupational exposures to the organophosphorous insecticides chlorpyrifos and profenofos in Egyptian cotton field workers. Int. J. Hyg. Environ. Health 218, 203–211. https://doi.org/10.1016/j.ijheh.2014.10.005.

Smith, J.N., Campbell, J.A., Busby-Hjerpe, A.L., Lee, S., Poet, T.S., Barr, D.B., Timchalk, C., 2009. Comparative chlorpyrifos pharmacokinetics via multiple routes of exposure and vehicles of administration in the adult rat. Toxicology 261, 47–58. https://doi.org/10.1016/j.tox.2009.04.041.

Stallones, L., Beseler, C., 2002. Pesticide poisoning and depressive symptoms among farm residents. Ann. Epidemiol. 12, 389–394. https://doi.org/10.1016/S1047-2797(01)00298-8.

Svensson, I., Waara, L., Cassel, G., 2005. Effects of HI 6, diazepam and atropine on soman-induced IL-1B protein in rat brain. Neurotoxicology 26, 173–181. https://doi.org/10.1016/j.neuro.2004.11.004.

Terry, A.V., 2012. Functional consequences of repeated organophosphate exposure: potential non-cholinergic mechanisms. Pharmacol. Ther. 134, 355–365. https://doi.org/10.1016/j.pharmthera.2012.03.001.

Vale, A., Lotti, M., 2015. Organophosphorus and carbamate insecticide poisoning. In: Handbook of Clinical Neurology, 1st ed. Elsevier B.V.. https://doi.org/10.1016/B978-0-444-62627-1.00010-X.

Voicu, V.A., Bajgar, J., Medvedovici, A., Radulescu, F.S., Miron, D.S., 2010a. Pharmacokinetics and pharmacodynamics of some oximes and associated therapeutic consequences: a critical review. J. Appl. Toxicol. 30, 719–729. https://doi.org/10.1002/jat.1561.

Voicu, V.A., Thiermann, H., Radulescu, F.S., Mircioiu, C., Miron, D.S., 2010b. The toxicokinetics and toxicodynamics of organophosphonates versus the pharmacokinetics and pharmacodynamics of oxime antidotes: biological consequences. Basic Clin. Pharmacol. Toxicol. 106, 73–85. https://doi.org/10.1111/j.1742-7843.2009.00486.x.

Wesseling, C., Keifer, M., Ahlbom, A., McConnell, R., Moon, J.D., Rosenstock, L., Hogstedt, C., 2002. Long-term neurobehavioral effects of mild poisonings with organophosphate and n-methyl carbamate pesticides among banana workers. Int. J. Occup. Environ. Health 8, 27–34. https://doi.org/10.1179/oeh.2002.8.1.27.

Witkin, J.M., Catlow, J.T., Wishart, G.N., Heinz, B.A., 2014. M1 and M2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly-acting. J. Pharmacol. Exp. Ther. 351, 448–456. https://doi.org/10.1124/jpet.114.216804.

Wright, L.K.M., Liu, J., Nallapaneni, A., Pope, C.N., 2010. Behavioral sequelae following acute diisopropylfluorophosphate intoxication in rats: comparative effect of atropine and cannabinomimetics. Neurotoxicol. Teratol. 32, 329–335. https://doi.org/10.1016/j.ntt.2009.12.006.

Yurumez, Y., Durukan, P., Yavuz, Y., Ikizceli, I., Avsarogullari, L., Ozkan, S., Akdur, O., Ozdemir, C., 2007. Acute organophosphate poisoning in university hospital emergency room patients. Intern. Med. 46, 965–969. https://doi.org/10.2169/internalmedicine.46.6304.