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Neurologic dysfunction and genotoxicity induced by low levels of chlorpyrifos

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ABSTRACT

Chlorpyrifos (CPF) is an organophosphorus cholinesterase inhibitor widely used as an insecticide. Neuro and genotoxicity of this agent were evaluated following daily subcutaneous injections at 0.1, 1 and 10 mg/kg or its vehicle to laboratory rats during one week, at the end of which somatosensory evoked potentials (SEP) and power spectrum of the electroencephalogram (EEGp) were recorded under urethane anesthesia. In another group of conscious animals, auditory startle reflex (ASR) was evaluated followed, after euthanasia, with measurements of plasma B-esterases, and genotoxicity with the alkaline comet assay (ACA) at the same CPF doses. The results indicated a CPF dose related inhibition of B-esterases. Enhanced inhibition of the ASR by a subthreshold pre-pulse was observed at all doses and ACA showed a significant higher DNA damage than vehicle controls in animals exposed to 10 mg/kg CPF. A trend to higher frequencies of EEGp and an increase in amplitude of the first negative wave of the SEP were found at all doses. The first positive wave of the SEP decreased at the CPF dose of 10 mg/kg. In summary, a shift to higher EEG frequencies and alterations of somatosensory and auditory input to the central nervous system were sensitive manifestations of CPF toxicity, associated with depression of B-esterases. The changes in electrical activity of the cerebral cortex and DNA damage observed at doses that do not elicit overt toxicity may be useful in the detection of CPF exposure before clinical signs appear.

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1. Introduction

Although pesticides in general and the insecticide chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl), CPF) in particular have improved agricultural productivity, the undesirable effects of their use on the environment and human health are increasingly degrading the sustainability of agriculture (Altieri, 1987). CPF is an organophosphorus (OP) acetylcholinesterase (AChE) inhibitor that affects nervous system functions by enhancing the availability of acetylcholine (ACh) at synaptic sites (Taylor, 1990), although non-cholinergic actions of this and other OP AChE inhibitors have been described (Checler, 1990; Johnson, 1975; Rao et al., 1999).

The widespread use of this insecticide is highlighted by the fact that CPF exposure has been detected in 86–96% of the U.S. population (Hill et al., 1995; Barr et al., 2005). Moreover, CPF metabolites have been found in 100% of urines tested from children in Ohio (Morgan et al., 2011).

Large amounts of CPF are used in agriculture in Argentina, estimated from import records of this insecticide at 6.8 million kilograms per year, and are used on various commodity crops and fruits, grains and vegetables of local consumption (SENASA, 2011). The ecological impact of the intensive use of this OP AChE inhibitor is reflected in persistent toxicity to soil organisms and runoff events into water bodies with invertebrate, anuran and fish kills (Marino and Ronco, 2005; Mugni et al., 2012; Jergentz et al., 2004; Loewy et al., 2011).

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Once incorporated to the organism, CPF is metabolized to its active form, chlorpyrifos oxon (Ma and Chambers, 1994; Khokhar and Tyndale, 2012) that phosphorylates the active site of AChE rendering it inactive. This leads to disruption of cholinergic neural transmission in the central, autonomic and peripheral nervous systems. Neurotoxic effects of CPF include cortical arousal, disorientation, alterations of the sleep-wakefulness cycle, fasciculations and convulsions. Peripheral autonomic effects include hypersecretion of salivary and lachrymal glands, hypertension, tachycardia, sweating, miosis and bronchorrhea. Respiratory insufficiency (Clegg and van Gemert, 1999; Barron and Woodburn, 1995) and convulsions (Jett, 2012) are observed at high doses.

Long term neurological sequelae of intoxication with OP AChE inhibitors have also been described (Rosenstock et al., 1991; Savage et al., 1988; Steenland et al., 1994).

Activity of B-esterases has been widely used for the evaluation of exposure to pesticides (Wheelock et al., 2008; Manzo et al., 2001). Chlorpyrifos and other organophosphorus (OP) and carbamate AChE inhibitors inhibit the activity of B-esterases following patterns characteristic of every agent (Taylor, 1990).

The electrical activity of the cerebral cortex has often been used for the evaluation of pesticides toxicity. EEG patterns and EEG power spectrum alterations in response to CPF exposure have been reported for a single exposure at doses of 10 and 40 mg/kg (Timofeeva and Gordon, 2002) but there is no information in the literature about effects of this pesticide at lower doses on EEG or at any dose on evoked electrical activity, a technique that has provided sensitive biomonitoring in the case of other neurotoxicants (Nagymajtenyi et al., 1998; Mwanza et al., 2012; Desi and Nagymajtenyi, 1988; Scremin et al., 2011).

Sensory inputs to the higher levels of the central nervous system are under modulatory control (sensory gating) that can be demonstrated by the phenomenon of pre-pulse inhibition (PPI), in which a sub-threshold sound pulse can inhibit the response to an alerting, high intensity auditory stimulus that follows it (Koch, 1999). This inhibition appears to be mediated by cholinergic muscarinic receptors at the level of the nucleus reticularis pontis caudalis (Jones and Shannon, 2000; Bosch and Schmid, 2006). It is then conceivable that OP AChE inhibitors like CPF might enhance PPI, a phenomenon known to occur with carbamate AChE inhibitors (Clark et al., 2005).

DNA damage and oxidative stress could mechanistically link pesticide exposures with a number of health outcomes observed in epidemiological studies (Muniz et al., 2008). Single cell gel electrophoresis (comet assay) has gained wide acceptance as a valuable tool in fundamental DNA damage and repair studies, genotoxicity testing and human biomonitoring. This assay was adapted to measure oxidized purines and pyrimidines by the incubation of the nucleoids with bacterial DNA repair enzymes. Formamidopyrimidine glycosylase (FPG) is used to detect oxidized purines, mostly 8-oxo-7,8-dihydroguanine (8-oxo-G) (Collins et al., 1997). A great variety of oxidized bases have been identified in nuclear DNA but 8-oxo-G is one of the most abundant and readily formed oxidized DNA lesions (Azqueta et al., 2009).

Chlorpyrifos may be administered by cutaneous, oral, intraperitoneal, inhalational, intravenous or subcutaneous routes. The lethal dose 50% (LD50) of this agent depends on the route of administration and animal species. For the subcutaneous (s.c.) route in rats it has been estimated at 147 mg/kg (WHO, 1975). The rationale for the use of subcutaneous administration of CPF in oil in the present experiments is based on the fact that it results in a slow sustained release of the pesticide into the systemic circulation which approximates most human dermal exposures (Ellison et al., 2011). Since exposures to CPF of agricultural workers or the population of urban centers within zones of pesticide drift often consist of repeated, daily exposure, the present work was designed to assess changes in neurological biomarkers of exposure and effect as well as possible genotoxicity following one week of daily s.c. doses of this agent. Doses ranging from approximately 1/1000 to 1/10 of the LD50 were chosen since they are usually devoid of overt toxicity, mimicking a scenario with no alerting clinical signals that may erroneously lead to the assumption of lack of danger.

The biomarkers of exposure (activity of B-esterases) and effects (EEG and comet assay) have been extensively used to assess exposure to pesticides in agricultural workers and at risk bystanders. See reviews by Reigart and Roberts (1999), Seppalainen (1975), and Valverde and Rojas (2009), respectively. Although somatosensory evoked potentials have not been used in humans for this purpose, it is a technique routinely used for the evaluation of patients with many neurological conditions and its implementation is well standardized. Thus the results of this study are readily translatable to the human population.

2. Materials and methods

2.1. Animal care and drug administration

Two batches of animals were used. Experiments including blood enzymatic activity, comet assays and auditory startle were performed in male Sprague-Dawley adult rats and experiments including power spectrum analysis of the electroencephalogram and somatosensory evoked potentials were performed in male Wistar adult rats. The reason for strain selection was based on the fact that Sprague-Dawley rats express more strongly the phenomenon of pre-pulse inhibition than Wistar rats and for this reason they were selected for the study arm including ASR. On the other hand, Wistar rats have been used in recent studies of cholinergic modulation of cortical somatosensory function (Alenda and Nunez, 2004, 2007) and in a previous study of somatosensory evoked potentials with dichlorvos, also an OP cholinesterase inhibitor (Papp et al., 1996). Thus, this strain was selected for the arm with EEG power spectrum and somatosensory evoked potentials to facilitate comparisons with previous work. B-esterase activity measurements and comet assays were carried out in the rats tested for the auditory startle response that were euthanized by decapitation. Those tests were not performed in Wistar rats, used in recording EEG and evoked potentials, in order to avoid the possible confounding effects of prolonged anesthesia with urethane, a known genotoxic agent (Schlatter and Lutz, 1990). The project received Institutional approval from the University of Rosario (Argentina) Medical School and all procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.

Animals were maintained in an environmentally controlled space, with lights on at 07:00 h and off at 19:00 h and air temperature between 20 and 25 °C. They were housed in polycarbonate cages with sawdust bedding, two animals to a cage, with access to rat chow and water ad libitum. Body mass was recorded daily and they were observed for signs of cholinergic toxicity (salivation, lachrymation, fasciculations, tremors, tail dorsiflexion, convulsions, and drop in body mass or rectal temperature). Chlorpyrifos (Supelco©, CHEM SERVICE, Inc. West Chester, PA) was dissolved in sunflower oil at the concentrations of 0.1, 1, and 10 mg ml^{-1} and administered subcutaneously at doses of 0.1, 1, and 10 mg/kg body mass once daily during 7 days to three groups of animals. One additional group was injected once daily with the same volume (1 ml/kg/body mass) of the CPF vehicle. Number of animals in the first batch for the drug vehicle, 0.1, 1 and 10 mg/kg doses were 10, 5, 9 and 4 and for the second batch 6, 5, 5 and 6 respectively. Body mass and rectal temperature were recorded daily.

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