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Cholinesterase inhibition and alterations of hepatic metabolism by oral acute and repeated chlorpyrifos administration to mice

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Abstract

Chlorpyrifos (CPF) is a broad spectrum organophosphorus insecticide bioactivated *in vivo* to chlorpyrifos-oxon (CPFO), a very potent anticholinesterase. A great majority of available animal studies on CPF and CPFO toxicity are performed in rats. The use of mice in developmental neurobehavioural studies and the availability of transgenic mice warrant a better characterization of CPF-induced toxicity in this species. CD1 mice were exposed to a broad range of acute (12.5–100.0 mg/kg) and subacute (1.56–25 mg/kg/day from 5 to 30 days) CPF oral doses. Functional and biochemical parameters such as brain and serum cholinesterase (ChE) and liver xenobiotic metabolizing system, including the biotransformation of CPF itself, have been studied and the no observed effect levels (NOELs) identified. Mice seem to be more susceptible than rats at least to acute CPF treatment (oral LD_{50} 4.5-fold lower). The species-related differences were not so evident after repeated exposures. In mice a good correlation was observed between brain ChE inhibition and classical cholinergic signs of toxicity. After CPF-repeated treatment, mice seemed to develop some tolerance to CPF-induced effects, which could not be attributed to an alteration of P450-mediated CPF hepatic metabolism. CPF-induced effects on hepatic microsomal carboxylesterase (CE) activity and reduced glutathione (GSH) levels observed at an early stage of treatment and then recovered after 30 days, suggest that the detoxifying mechanisms are actively involved in the protection of CPF-induced effects and possibly in the induction of tolerance in long term exposure. The mouse could be considered a suitable experimental model for future studies on the toxic action of organophosphorus pesticides focused on mechanisms, long term and age-related effects.

Keywords: Organophosphate insecticides; Chlorpyrifos; Cholinesterase; Oral acute and repeated toxicity; Metabolism; Mouse

Abbreviations: AChE, acetylcholinesterase; AcThCh, acetylthiocholine; CE, carboxylesterase; ChE, cholinesterase; CPF, chlorpyrifos; CPFO, chlorpyrifos-oxon; CYPs, cytochrome P450 isoforms; DTNB, dithiobisnitrobenzoic acid; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; GSH, reduced glutathione; HLM, human liver microsomes; iso-OMPA, tetraisopropyl-pyrophosphoramide; LOAEL, lowest observed adverse effect level; MLM, mouse liver microsomes; NOEL, no observed effect levels; OPT, organophosphorothionate; PON, paraoxonase; PYRI, 3,5,6 trichloro-2-pyridinol; RLM, rat liver microsomes; SLUD, salivation; lacrimation; urination and defecation; TST, testosterone

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1. Introduction

Chlorpyrifos (CPF), a broad spectrum organophosphorothionate (OPT) insecticide, is extensively used in agricultural and household insect control, because of its moderately acute mammalian toxicity, compared to many others pesticides (Cochran et al., 1995; Pope et al., 1991; Pope, 1999; Richardson, 1995). However, the potential long term health effects associated with human exposure to low CPF levels have been the subject of increasing concern in the last years (Ray, 1998, 2000). Subtle, mainly cognitive, long term effects due to chronic professional exposure to low OPT levels have been reported in humans (Beach et al., 1996) not only following attacks of acute cholinergic episodes (Jamal et al., 2002); furthermore, covalent binding to novel protein targets that are sensitive to low levels of OPTs in the brain have been described (Richards et al., 1999). The major debate over potential long term OPT effects is focused on children. Indeed, epidemiological data (Eskenazi et al., 1999) indicated that the urinary concentration of the primary CPF metabolites in children were up to two times higher than levels observed in comparable studies with adults (Gurunathan et al., 1998). In addition, neurodevelopmental effects, due to OPT pre- or early postnatal exposure, have been reported both in experimental animals (Aldridge et al., 2005; Ricceri et al., 2003; Ricceri et al., 2006) and humans (Berkowitz et al., 2004). Because of this growing concern, the US Food Quality Protection Act (FQPA, 1996) focused attention on the possible higher susceptibility of children with respect to adults, due to exposure to neurotoxic pesticides, such as CPF, and asked for the production of data on the mechanism of age-related effects.

CPF is readily bioactivated *in vivo* by cytochrome P450 isoforms (CYPs) to its active metabolite, chlorpyrifos-oxon (CPFO), a very potent inhibitor of acetylcholinesterase (AChE), responsible for the hydrolysis of acetylcholine, the key molecule in the control of cholinergic transmission in the central and peripheral nervous system. During OPT desulfuration, activated sulphur atoms are formed, binding irreversibly to the CYP catalysing the reaction, resulting in time-dependent reduction of the related enzymatic activity (Halpert et al., 1980; Sultatos, 1994). The P450-mediated dearylation of the parent insecticide can detoxify CPF to 3,5,6 trichloro-2-pyridinol (PYRI), thus reducing the *in vivo* toxicity of CPF (Chambers and Chambers, 1990) (Fig. 1). In addition, both in the liver and in plasma, CPFO interacts with B esterases, i.e. carboxylesterases (CEs) that act as "molecular scavengers" by binding stoichiometrically to the oxon, and with A-esterases

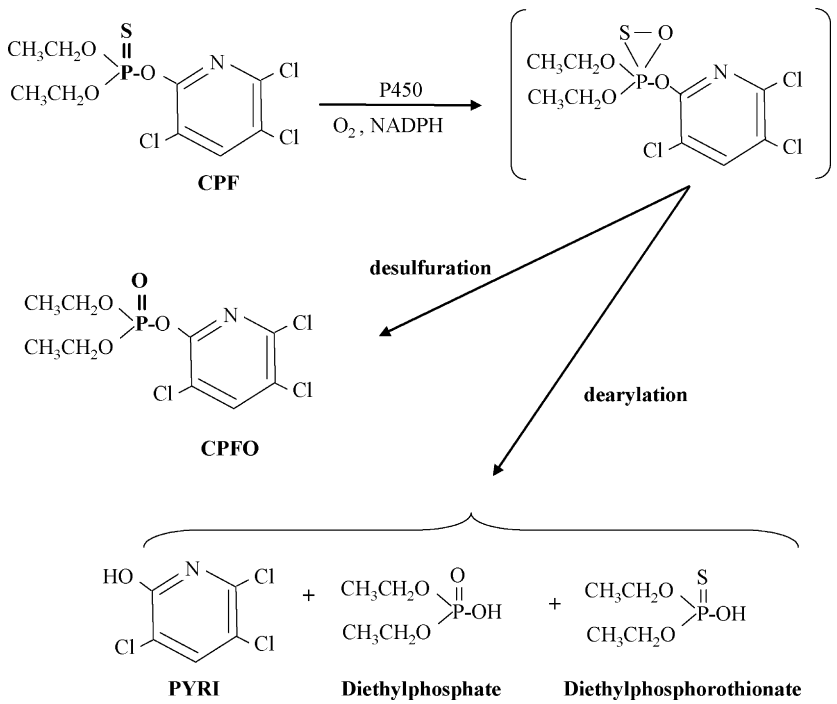

Fig. 1. Chlorpirifos major metabolic pathways.