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Foetal and neonatal exposure to chlorpyrifos: Biochemical and metabolic alterations in the mouse liver at different developmental stages

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ABSTRACT

The mechanisms implicated in the age-related toxicity, including its neurobehavioral effects after subtoxic developmental exposure to chlorpyrifos (CPF), a widely used insecticide, have not been fully elucidated yet. With the aim of investigating whether metabolic differences during ontogeny could account for the age-related susceptibility to CPF, we examined the developmental time-course of hepatic metabolizing enzymes and CPF metabolism in a cohort of mice exposed either prenatally (gestational day 15–18) and/or postnatally (postnatal day (PND) 11–14) to CPF at doses which were previously reported to induce neurobehavioural alterations, in the absence of brain acetyl-cholinesterase inhibition. Testosterone hydroxylase activity, CPF *ex vivo* biotransformation, glutathione content, as well as aromatase activity were determined in the liver of control and treated male and female mice at PND0, 9, 15 and 150.

In control mice most Cyp activities were detectable and progressively increased up to PND15. In newborn control mice CPF bioactivation was much higher than the Cyp-catalysed detoxication, negligible at birth, indicating a possible increased susceptibility to CPF-induced effects in newborn mice. Detoxication rapidly increased with age, so that Cyp-related metabolic features cannot explain the higher susceptibility of juvenile mice. The observed age-dependent metabolic picture was partially altered by CPF prenatal treatment. Following *in utero* exposure CPF detoxifying capability was enhanced at birth and reduced at PND15, when CPF-oxon formation was slightly increased. No effects were evident at adulthood. Prenatal dosing was more effective in causing metabolic alterations than CPF postnatal treatment; no potentiation was observed in mice experiencing pre- plus post-natal CPF administration. Both *in utero* and postnatal CPF exposure decreased aromatase activity by 50% at PND9 and 15; this effect together with the presence of higher levels of the sex-specific Cyp2c activity at adulthood in male mice may suggest the occurrence of long-lasting impairment in the expression of hepatic Cyps under hormonal regulation. Altogether, the alterations in CPF Cyp-mediated biotransformation caused by perinatal CPF exposure seem not sufficient *per se* to explain the reported vulnerability of developing central nervous system to this insecticide, which can be due also to the parent compound itself or to the activation of different toxicological pathways. The hypothesis that observed effects on aromatase and sex-specific Cyp activity may be associated with a possible interference with the long-term alterations in sex-specific behavioural pattern deserves further investigation.

1. Introduction

Chlorpyrifos (CPF), an organophosphorothionate (OPT) insecticide, has been extensively used for many years in agriculture on a wide range of crops and in residential applications for pest control (Eaton et al., 2008; Testai et al., 2010). Infants and children may experience higher levels of exposure (Adgate et al., 2001; Fenske et al., 2002), due to its presence as a residue in food, to its possible accumulation on furniture surfaces and toys after household application or "take home" exposure for the farm-worker families. Indeed, by using urinary biomarkers for OPT exposure, some epidemiological studies suggested that the urinary concentrations of the primary CPF metabolite were up to 2-fold higher in children than levels observed in comparable studies with adults (Eskenazi et al., 2007). In addition, children have been considered as a susceptible subpopulation due to CPF-induced higher systemic toxicity reported in neonates and weanlings rats than in adult animals (Zheng et al., 2000). For these reasons, in 2002 USEPA

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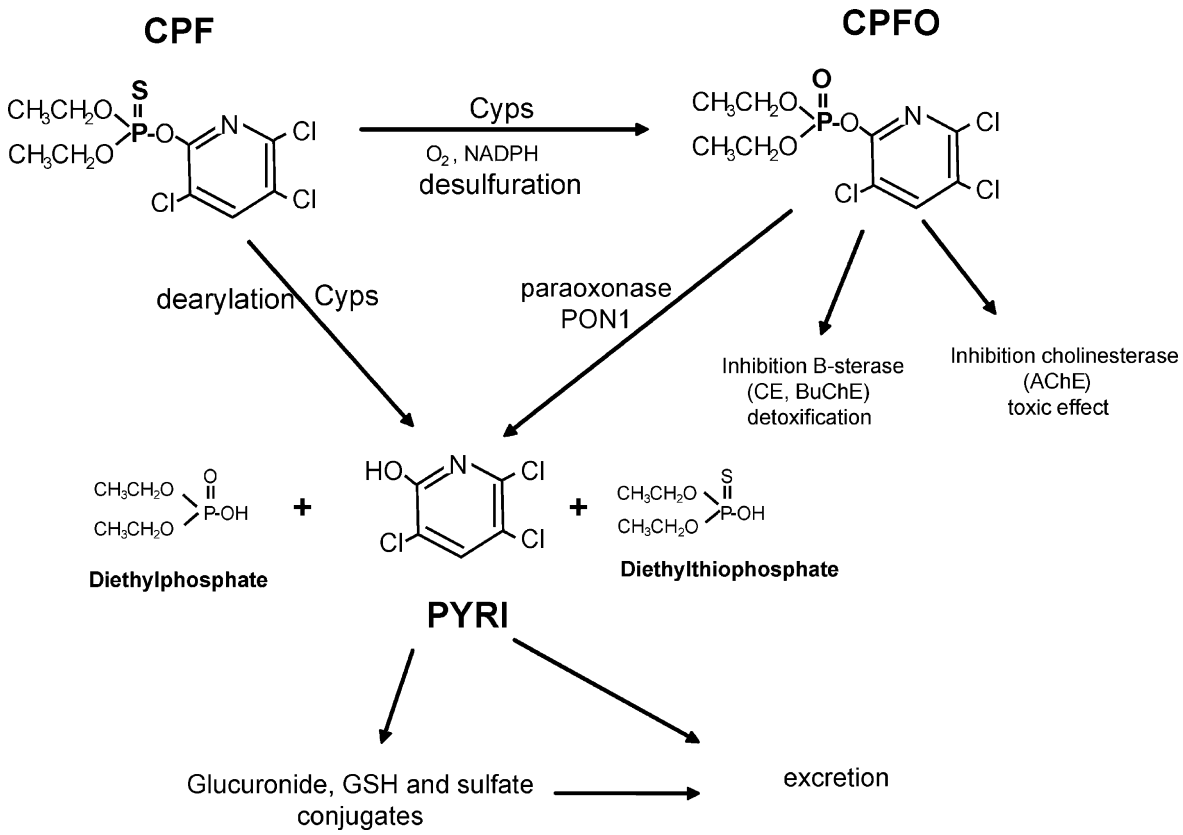

Fig. 1. CYP-dependent metabolism of chlorpyrifos (CPF), CPFO = chlorpyrifos-oxon; PYRI = 3,5,6-trichloropyrin-2-ol.

banned all home-owner uses and adopted the decision to reduce the non-agricultural uses to less than 3% (EPA, 2002). More recently, CPF perinatal exposure has been reported to induce a number of behavioral alterations attributed to neurochemical effects in the developing brain (Aldridge et al., 2005; Ricceri et al., 2006; Richardson and Chambers, 2005; Venerosi et al., 2006, 2009), other than cholinergic impairment (Slotkin et al., 2006).

The mechanisms underlying the higher susceptibility of young and developing rodents to CPF have not been fully clarified yet (Testai et al., 2010). Although interactions with targets with different susceptibility in developing organs have to considered, the age-related sensitivity to CPF effects has been partially attributed to toxicokinetic and biotransformation differences (EPA, 2008), leading to different levels of formation of chlorpyrifos-oxon (CPFO), the actual inhibitor of acetylcholinesterase (AChE), and/or to its detoxication metabolites. The desulfuration reaction to CPFO is mediated by cytochrome P450, which can catalyse also CPF dearylation to the inactive 3,5,6-trichloro-2-pyridinol (PYRI) (Fig. 1). Additional detoxifying pathway involving A and B esterases take place in the liver and in plasma (Karanth and Pope, 2000); moreover, CPF and CPFO undergo a very extensive phase II mediated metabolic detoxification leading to GSH-derived conjugates as well as O- and S-glucuronides in human hepatocytes (Choi et al., 2006).

The age-dependent CPF biotransformation has been investigated in rats (Atterberry et al., 1997; Moser et al., 1998; Moser and Padilla, 1998; Marty et al., 2007), but no data is available on CPF biotransformation in mice during developmental stages, and only information on protein expression are available on the murine ontogenetic profile of drug metabolizing enzymes (Choudhary et al., 2005; Hart et al., 2009). Despite this, the great majority of the neurodevelopmental studies have been carried out on mice: hence information on this specie should be available for data interpretation.

The aim of the present study was to investigate in a murine model of perinatal CPF exposure, whether metabolic differences could account for the age-related susceptibility to CPF. To this aim we evaluated the developmental time course of hepatic metabolizing enzymes indicative of Cyp-mediated reactions and involved in CPF metabolism in livers collected from a cohort of mice showing long-term sex-specific neurobehavioural alterations associated to CPF exposure (Ricceri et al., 2006; Venerosi et al., 2006, 2009), in the absence of significant brain AChE inhibition. The chosen exposure windows, namely the late gestational phase (gestational day – GD15–18) and the late neonatal age (postnatal day – PND11–14), are characterized by different CNS maturational events and represent critical phases of susceptibility to CPF in rodents (Garcia et al., 2003), whereas PND150 is included to identify long-term effects. Chlorpyrifos exposure of pups during gestation was demonstrated by the significant decrease of serum AChE activity (Ricceri et al., 2006).

Testosterone hydroxylase (TST-OH) as a multi-marker of Cyp-induced reactions and CPF *ex vivo* Cyp-mediated biotransformation (CPFO and PYRI formation) as indicative of the capacity to metabolize an additional CPF administered dose were determined in both control and CPF-exposed male and female mice at different age points (PND0, 9, 15, and 150). Aromatase (AR) activity was also analyzed to verify whether an alteration in hepatic steroid metabolism could possibly correlate with the previously reported sex-dependent behavioral changes observed in the same cohort of mice.

2. Materials and methods

2.1. Chemicals

CPF (purity 98.8%), CPFO (purity 95%), 3,5,6-trichloropyrin-2-ol (PYRI) (purity 95%) were purchased from Chem Service (West Chester, PA). Roche GmbH