IDENTIFICATION OF HUMAN CYTOCHROME P450 ISOFORMS IN PHOSMET BIOACTIVATION AND METABOLIC INTERACTIONS WITH CHLORPYRIFOS

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Background. Organophosphate Pesticides (OPTs) are a class of compounds used in agriculture and on animal for pest control. Human exposure to OPTs is associated to occupational activities or due by residual on food and in water for the general population. Their oxidative desulfuration catalyzed by cytochrome P450 (CYP), causes the formation of a neurotoxic metabolite, called oxon, known to inhibit Acethylcolineterase (AChE). The potential correlation between exposure to OPTs and neurodegenerative diseases and/or neurodevelopmental effects has been extensively debated in the last years. In this work we studies the human metabolism of Phosmet (Pho) an OPT for which data are not available in a concentrations range of 0.5-300 μM, representative on human exposure. Moreover, since human may be co-exposed to different OPTs, possible metabolic interactions between Pho and Chlorpyrifos (CPF) was also investigated.

Methods. Pho metabolism was studied using an integrated *in vitro* approach with single human recombinant CYP isoforms, Human Liver Microsomes (HLM) and Human Intestine Microsomes (HIM), determining the kinetic parameters (Vmax, Km and Cli) and the activity of chemical inhibitors specific CYPs on Phosmet-oxon (PhOx) formation. To analyze and quantify the metabolite formation an *ad hoc* HPLC method was set up.

Results. The characterization of the reaction in single human recombinant CYPs evidenced the main involvement of CYP2C family with a Cli ranking of 2C18>2C19>2B6>2C9>1A1>1A2>2D6>3A4>2A6. The major involvement of CYP2C was confirmed by using specific chemical inhibitors in HLM where a single typical kinetic curve, while in HIM a biphasic reaction was evidenced, due to the presence in the gut of the two CYPs 2C and 3A4. Furthermore, Pho could efficiently inhibit both CPF bioactivation and detoxication, while the opposite is not relevant at the actual exposure levels.

Conclusions. Considering the average human hepatic CYP content, CYP2C19 contributed for the great majority (60%) at relevant (low) exposure concentrations, while CYP2C9 (33%) and CYP3A4 (31%) were relevant at high substrate concentration. Accounting for ¼ of that measured in the liver, the role of the gut pre-systemic bioactivation was also relevant. The prominent role of CYP2C in oxon formation was not shared by other OPTs for which CYP1A2 and CYP2B6 were the most active CYPs in forming the corresponding oxon at low exposure concentration. Consequently, the peculiar metabolic interaction between Pho and CPF bioactivation and detoxification depends on the different isoform-specific Pho bioactivation when compared to other OPTs.