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CYP superfamily perturbation by diflubenzuron or acephate in different tissues of CD1 mice

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Abstract

This work aimed to investigate whether the insecticide acephate (125 or 250 mg/kg b.w.) or diflubenzuron (752 or 1075 mg/kg b.w.), two of the most widely used pesticides worldwide, impairs CYP-linked murine metabolism in liver, kidney and lung microsomes after repeated (daily, for three consecutive days) i.p. administration. The regio- and stereo-selective hydroxylation of testosterone was used as multibiomarker of different CYP isoforms. Both gender and tissue specific effects were observed. Lung was the most responsive tissue to induction by lower diflubenzuron dose, as exemplified by the marked increase of testosterone 7α-hydroxylation (CYP2A) (up to 13-fold) in males. Higher dose produced a generalized inactivation. At the lower dose acephate induced 6β- (CYP3A1/2, liver) as well as 2β- (CYP2B1/2, kidney) hydroxylase activities (~5 and ~4-fold increase, respectively) in males. In females, a marked suppression of the various hydroxylations was observed. At 250 mg/kg of acephate, animals did not survive. Induction of the most affected isoforms was sustained by immunoblotting analysis. Corresponding human CYP modulations might disrupt normal physiological functions related to these enzymes. Furthermore, the co-mutagenic and promoting potential of these pesticides, phenomena linked to CYP upregulation (e.g. increased bioactivation of ubiquitous pollutants and generation of oxygen free radicals) are of concern for a more complete definition of their overall toxicological potential.

Keywords: Acephate; Diflubenzuron; CYP; Testosterone hydroxylase; Pesticides

1. Introduction

The greatly increased use of pesticides during recent decades has led to improvements in the quantity/quality of human nutrition, but has also given rise to environmental and public health risks. Whereas the acute effects of exposure to high doses of pesticides are well known (from certification procedures and reports of poisoning epidemics, occupational accidents from suicide attempts), the long-term effects of lower exposure levels remain controversial.

The benzoylphenylurea insecticide diflubenzuron (*N*-{[(4-chlorophenyl) amino]carbonyl}-2,6-difluorobenzamide) is an acridicide that is used throughout the world for reduced agent/area and barrier treatments, inhibiting the synthesis of new chitin in target organisms. Toxicological investigations showed a cytotoxic activity of this pesticide in CHO-K1 cell culture (Bayoumi et al., 2003). Diflubenzuron induces in vitro cytotoxic and cell transforming activities on BALB/c 3T3 cells (Perocco et al., 1993), enhances numbers of pluripotential stem cells both in vivo and in vitro (Jenkins et al., 1993), and alters tumor growth in both C57BL/6 mice with B16 melanomas (Jenkins et al., 1984, 1986; Hofs and McVie, 1991) and AKR mice with skin tumors (Jenkins et al., 1984). This pesticide showed negative

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results in a host-mediated transplacental carcinogen assay (Quarles et al., 1980) in a mouse micronuclei test, and in the Ames *Salmonella* reverse mutation test (Macgregor et al., 1979). In vivo long-term co-carcinogenicity bioassays were negative in both mice and rats of either sex (National Pesticide Information Retrieval System, Diflubenzuron, 1987). Conflicting data exist on the in vitro effects of diflubenzuron on the CYP superfamily of isoenzymes: it has been reported to be a potent inhibitor of TCDD-induced CYP1A1 expression in HepG2 cells (Ledirac et al., 2000), but also to bind with the Ah receptor responsible for CYP1A1 induction in hepatic and epidermal cells (Delescluse et al., 1998). In vivo data on the effects of diflubenzuron on the CYP-dependent microsomal metabolism are currently lacking.

Acephate (*O*,*S*-dimethyl *N*-acetylphosphoramidothiolate), which is widely used for the control of agricultural and domestic pests, is one of the top ten organophosphorous pesticides sold around the world. Much literature exists on the effects of this insecticide on both in vitro and in vivo biological tests. Short-term genotoxicity assays that tested positive include mutations in the Ames test (Waters et al., 1982), reverse expression of β-lactamase gene in *Salmonella typhimurium* JK947 (Hour et al., 1998), mutagenesis in yeast (Jones et al., 1984), unscheduled DNA synthesis in mammalian cells (Simmon et al., 1978), sister chromatid exchange in mammalian cells in vitro (Jones et al., 1984), HGPT in mammalian cells (Carver et al., 1985), point mutations; mitotic recombination and chromosomal malsegregation in *Saccaromyces cerevisiae* (DeKergommeaux et al., 1983), mammalian chromosomal aberrations; dominant lethals or sperm abnormalities in vivo (DeKergommeaux et al., 1983), mammalian micronucleus test in vivo (Jena and Bhunya, 1994), mutations in higher plants (Behera and Bhunya, 1989), and genotoxicity in vivo (COMET assay) (Rahman et al., 2002). Negative findings were reported for mutagenicity in *Neurospora crassa* (Brockman et al., 1984); the recessive lethal test in *Drosophila melanogaster* (Lee et al., 1983) and unscheduled DNA synthesis in human fibroblasts (Mitchell et al., 1983). Acephate is classified as a strong mutagenic agent in genetic activity profiles (Waters et al., 1982). Transforming activity in BALB/c 3T3 cells and in vivo co-carcinogenic potential (linked to a metabolic burst) have also been reported (Perocco et al., 1996; Paolini et al., 1997). Acephate is classified as a possible human carcinogen, based on increased incidence of hepatocellular carcinomas and adenomas in female mice (US Environmental Protection Agency's Integrated Risk Information System).

To help better define the toxicological profiles of these insecticides, we investigated whether diflubenzuron and acephate can induce peculiar effects mediating deleterious outcomes of xenobiotics, such as co-mutagenicity/co-carcinogenesis (Lubet et al., 1992; Paolini et al., 1995, 1999). We therefore analyzed the ability of diflubenzuron and acephate to impair CYP-supported metabolism in murine liver, kidney and lung, using the regio- and stereo-selective hydroxylation of testosterone as a multibiomarker of effect.

2. Materials and methods

2.1. Chemicals

Diflubenzuron (*N*-{[(4-chlorophenyl) amino]carbonyl}-2,6-difluorobenzamide) and acephate (*O*,*S*-dimethyl *N*-acetylphosphoramidothiolate) (see Fig. 1) were purchased at 99.5% chemical purity from Lab Service (Bologna, Italy). $NADP^+$, NADPH, 16α-hydroxytestosterone, corticosterone, testosterone, and 4-androsten-3,17-dione were all purchased from Sigma Chemical Co. (St. Louis, MO); glucose 6-phosphate and glucose 6-phosphate dehydrogenase were from Boehringer-Mannheim (Germany); HPLC-grade methanol, tetrahydrofuran and dichloromethane from Labscan Ltd. (Co. Dublin, Ireland); 7α-, 6β- and 16β-hydroxytestosterone from Steraloids (Wilton, NH); 6α-, 2α- and 2β-hydroxytestosterone were a generous gift from Dr. P.G. Gervasi (CNR, Pisa, Italy). All other chemicals and solvents used were of the highest purity commercially available.

2.2. Animal treatment and preparation of hepatic subcellular fractions

Male and female Swiss albino CD1 mice (Harlan-Italy, Milan, Italy) weighing 26–30 g were housed under controlled conditions (12 h light–dark cycle, 22 °C, 60% humidity). Throughout the study, they were treated in accordance with *National Academy of Sciences* guidelines. They were fed with rodent chow and had tap water ad libitum. Insecticides were administered at scalar doses from 50% of the LD_{50}. Diflubenzuron was dissolved in corn oil and administered (i.p.) in repeated doses (752 or 1052 mg/kg b.w., daily for three consecutive days). Acephate was dissolved in corn oil and administered (i.p.) in repeated doses (125 or 250 mg/kg b.w., daily for three consecutive days). Controls received vehicle only (corn oil), under the same conditions. The

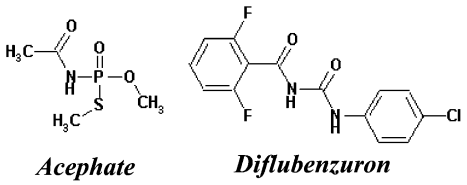

Fig. 1. Chemical structure of diflubenzuron and acephate.