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Study on the toxicity of phenolic and phenoxy herbicides using the submitochondrial particle assay

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Abstract

A simple and rapid in vitro toxicological assay, utilizing submitochondrial particles (SMP), has been used to evaluate the toxic effects of fifteen herbicides belonging to the phenol and phenoxyalkanoic acid chemical classes.

The SMP assay allows the quantitative evaluation of the toxicity of compounds with different mechanisms of action: uncouplers, inhibitors of the enzyme complexes involved in reverse electron transfer and in oxidative phosphorylation and chemicals that alter the membrane structure.

The two groups of herbicides showed different levels of toxicity. For phenol derivatives, EC_{50} values ranged from 0.16 μM (ioxynil) to 6.7 μM (2,4-dinitrophenol), whereas for phenoxy herbicides EC_{50} values ranged from 21 μM (2,4,5-trichlorophenoxyacetic acid, 2,4,5-T) to 110 μM (4-chloro-2-methylphenoxyacetic acid, MCPA). On the average, the toxicity of phenolic compounds is greater than that of phenoxyalkanoic acids by two orders of magnitude.

Quantitative structure–activity relationships (QSAR) were developed between EC_{50} values and various molecular descriptors. The results suggest the existence of different mechanisms of action for the two classes of compounds. The findings obtained for phenolic herbicides are consistent with a protonophoric uncoupling mechanism, whereas for phenoxy herbicides a non-specific mode of action at membrane level can be hypothesized.

Keywords: Submitochondrial particles; Herbicides; Dinitrophenols; Hydroxybenzonitriles; Phenoxyalkanoic acids; Toxicity; Quantitative structure–activity relationship

Abbreviations: μ, dipole moment; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 2,4,5-TP, 2-(2,4,5-trichlorophenoxy)propionic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4-DB, 4-(2,4-dichlorophenoxy)butanoic acid; 2,4-DP, 2-(2,4-dichlorophenoxy)propionic acid; DNOC, 2-methyl-4.6-dinitrophenol; DNP, 2.4-dinitrophenol; EC_{50}, effective concentration that inhibits reverse electron transfer by 50%; E_{HOMO}, energy of the highest occupied molecular orbital; E_{LUMO}, energy of the lowest unoccupied molecular orbital; $\log K_{ow}$, logarithm of the *n*-octanol/water partition coefficient; MCPA, 4-chloro-2-methylphenoxyacetic acid; MCPB, 4-(4-chloro-2-methylphenoxy)butanoic acid; MCPP, 2-(4-chloro-2-methylphenoxy)propionic acid; pK_a, acid dissociation constant; QSAR, Quantitative structure–activity relationship; RET, reverse electron transfer; SMP, submitochondrial particles; V_{mc}, molecular volume.

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1. Introduction

A wide number of environmental pollutants exert their toxic action by interfering at different levels with mitochondrial respiration, a fundamental step of energy metabolism in eukaryotic organisms. Mitochondria have been recognized as subcellular targets of many xenobiotic compounds; as a consequence, toxicity endpoints related to respiratory functions have been widely used in recent years to assess the adverse effects of various toxicants and to investigate the mechanisms of their action by means of suitable in vitro systems. Submitochondrial particles (SMP) proved to be very useful to this purpose. They are constituted by closed vesicles of inner membrane obtained by sonic disruption of mitochondria, and maintain the capacity to perform electron transport and oxidative phosphorylation. In particular, SMP from beef heart mitochondria are very stable and, if preserved at low temperature, maintain their activity for relatively long times. This, together with the direct contact of SMP with toxicants due to the absence of an outer membrane, makes SMP excellent in vitro biosensors for determining the adverse effects of xenobiotics (Blondin et al., 1987; Blondin et al., 1989; Knobeloch et al., 1990; Oakes and Pollack, 1999; Argese et al., 2001; Gustavson et al., 2002) and for investigating the molecular mechanism of toxic action from quantitative structure–activity relationships (QSAR) (Todeschini et al., 1996; Gustavson et al., 1998; Argese et al., 2002).

The SMP assay is based on the process of reverse electron transfer (RET). The rate of NADH formation during this process is monitored spectrophotometrically at 340 nm, and the decrease caused by the presence of toxicants is used as toxicity endpoint. The assay responds to compounds that act by different modes of action, resulting in the same observable effect, that is the slowing down of the reverse electron transfer and, consequently, of the NADH formation. The assay is sensitive to uncouplers, to inhibitors of the enzymatic complexes involved in electron transfer (succinate dehydrogenase, NADH dehydrogenase) and oxidative phosphorylation (ATP synthase) and, furthermore, to agents that alter the membrane integrity (Knobeloch et al., 1990; Argese et al., 2001, 2002). Since the orientation of the mitochondrial membrane in SMP is inverted with respect to that in intact mitochondria, these enzymatic complexes are directly exposed to the action of toxicants, thus conferring to the assay the characteristics of rapid response and of increase of sensitivity.

Toxicity is quantified in terms of EC_{50}, that is the toxicant concentration that reduces NADH formation rate by 50%. The assay is simple, rapid and repeatable (Doherty and Gustavson, 2002); previous studies have shown that the response is comparable to that of the most commonly used bioassays for acute aquatic toxicity, which utilize both in vitro systems and aquatic organisms, such as algae, invertebrates and fishes (Argese et al., 1998).

The names and structures of the 15 different pesticides examined in this study are reported in Fig. 1.

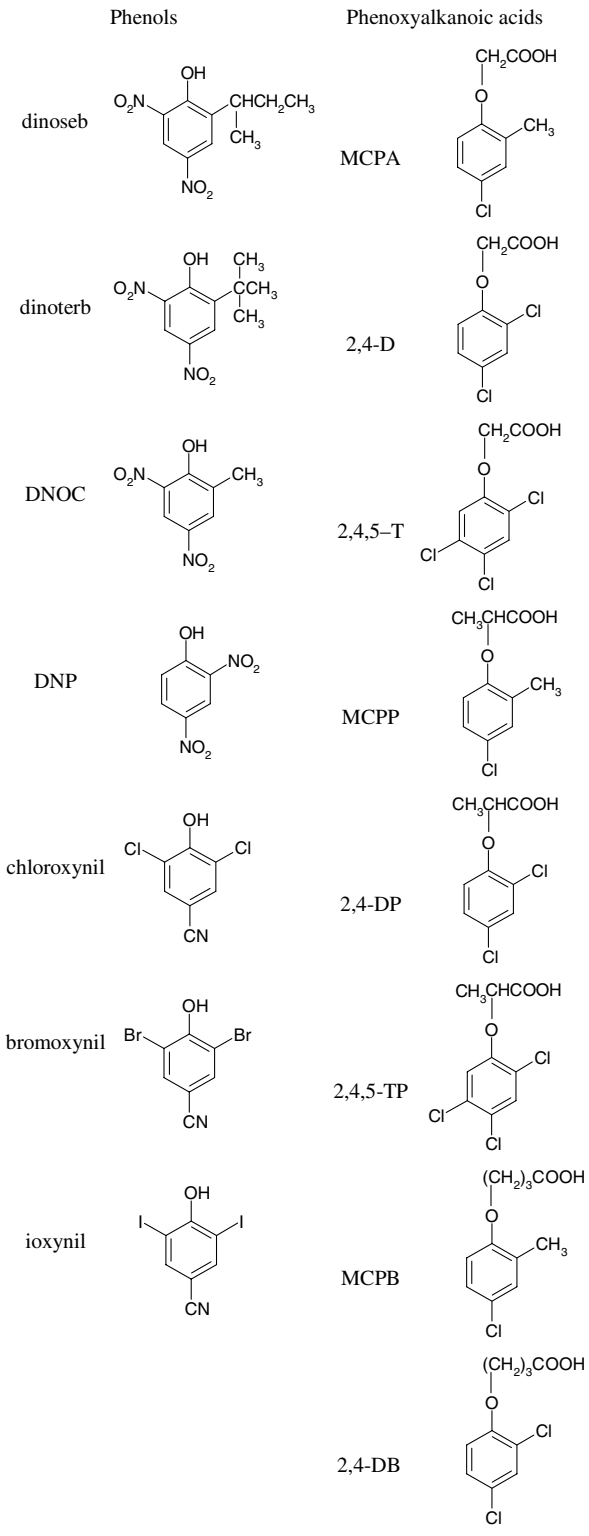

Fig. 1. Names and structural formulas of the examined herbicides.