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Toxicity Evaluation of 4-Chloro-2-Methylphenoxyacetic Acid by Microtox and Comparison with FETAX

C. Vismara,[1] C. Rossetti,[2] E. Bolzacchini,[2] M. Orlandi,[2] A. Luperini,[3] G. Bernardini[1]

[1]Department of Biology, University of Milan, 26 Via Celoria, I-20133 Milano, Italy
[2]Department of Environmental Sciences, University of Milan, 15 Via Emanueli, I-20126 Milano, Italy
[3]ENEA, 2 P.le Morandi, I-20121 Milano, Italy

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Inasmuch the effects of xenobiotics on living organisms are, to a large extent, species-specific, to quantify the hazard of toxicants it would be necessary to use a battery of bioassays, representative of the different trophic levels in the ecosystem. At present, a wide range of bioassays is available for biological monitoring and toxicity assessment; one among so many is the simple, rapid and sensitive Microtox assay. The Microtox system was originally developed to assess the toxic effects of complex industrial effluents (Bulich 1984) and, subsequently, it was extended to determine the toxicity of aquatic pollutants, waste water, fossil-fuel process waters and numerous other chemicals (Ribo and Kaiser 1987).

In this paper, we have investigated the toxicity of the 4-chloro-2-methylphenoxyacetic acid (MCPA) containing compounds using Microtox assay. Moreover, we have verified a possible mutual relation between the results of FETAX (Frog Embryo Teratogenesis Assay-*Xenopus*) performed in our laboratory and the bioluminescence inhibition of *Photobacterium phosphoreum* using the Microtox test. FETAX, thanks to its three endpoints (i.e., mortality, malformation and growth inhibition), is a powerful and reliable bioassay that makes use of *Xenopus* embryos (Dumont *et al.* 1983; Bantle *et al.* 1989; Vismara et al. 1993; Bernardini *et al.* 1994; Presutti *et al.* 1994).

MATERIALS AND METHODS

Light production from luminescent bacteria was measured in a

Correspondence to: C. Vismara

Microtox Model 500 and by following the procedures detailed in the Microtox manual. The freeze-dried bacteria, reconstitution solution (organic-free distilled water), and diluent (a solution containing 20 g/L NaCl to provide osmotic stability for the marine bacterium) were supplied by Microbics Corporation, Carlsbad, California. The commercial formulation of MCPA (Erbitox E30) was supplied by Società Italoamericana Prodotti Antiparassitari, Roma, Italy; technical grade MCPA Na salt, phenol and chlorocresol, by Sigma Chemical Co., St. Louis, Missouri, USA. The MCPA Na salt was purified by crystallization and acid-base purification starting from 97% MCPA (Fluka Chemie AG, Buchs, Switzerland), and its purity concerning phenol and chlorocresol presence was checked by HPLC (Bernardini et al. 1994); purified MCPA Na salt resulted to contain less than 0.04% (w/w) of phenol and less than 0.004% (w/w) of chlorocresol. The initial concentrations (to be diluted following Microbics instruction manual specifications) were determined with a range finding test. They were obtained from stock solutions prepared fresh for each experiment. The data were plotting log-log of sample concentration vs gamma, the ratio of light lost to the light remaining (Bulich et al. 1981). The toxicity endpoints were the 5 and 15 min median effective concentration (EC_{50}) for inhibition of luminescence.

RESULTS AND DISCUSSION

The acute toxicity of MCPA (purified, technical grade and the commercial formulation for agriculture) and that of two common contaminants (chlorocresol and phenol) were determined with the Microtox procedure (Table 1). Technical grade MCPA Na salt and Erbitox E30 were more toxic than the purified compound. This observation becomes more relevant if one takes into account that technical grade MCPA Na salt and Erbitox E30 contain approximately 96 and 24% of the phytoactive compound (i.e., MCPA salt), respectively. Therefore, we have compared the MCPA containing compounds for their MCPA content: in other words, 100 mg of technical grade MCPA Na salt and 100 mg of Erbitox E30 contain about 96 and 24 mg of purified MCPA salt, respectively. Accordingly, we have transformed the results of' Table I in those of Table 2. If the toxicity depends only on the content of the phytoactive substance, i.e.

Table 1. Median effective concentrations together with their SD determined with the Microtox system (at 5 and 15 min).

Test compound	EC_{50} (mg/L, 5 min)	EC_{50} (mg/L, 15 min)
purified MCPA Na	299 ± 47	248 ± 52
technical grade MCPA Na	149 ± 83	121 ± 63
Erbitox E30	289 ± 39	270 ± 38
phenol	22	25
chlorocresol	1.91±0.94	1.8±0.83

Table 2. Technical grade and Erbitox E30 are compared to the pure compound for the amount of MCPA Na salt contained. Technical grade contains about 96% of the active molecule, while Erbitox E30 contains 24%.

Test compound	EC_{50} (mg/L, 5 min)	EC_{50} (mg/L, 15 min)
purified MCPA Na	299 ± 47	248 ± 52
technical grade MCPA Na	143 ± 80	116 ± 60
Erbitox E30	69 ± 9	64 ± 9

Table 3. LC_{50}, TC_{50} (median Teratogenic Concentration) and MCGI obtained by FETAX procedure (Bernardini *et al.*, unpublished results).

Test compound	LC_{50} (mg/L)	TC_{50} (mg/L)	MCGI (mg/L)
purified MCPA Na	3607	2691	1000<MCGI<2000
phenol	178	141	<25
chlorocresol	13.4	12.1	<2.5

MCPA salt, one would expect to obtain, after transformation, similar results and this was not the case (Table 2). These large differences can be ascribed to the presence in the commercial formulation and in the technical grade of contaminants with higher toxicity. This hypothesis is consistent with previous findings (Bernardini *et al.*, 1994) showing that such products are contaminated with phenol and chlorocresol. Phenol and chlorocresol are indeed more toxic than purified MCPA by 1 and 2 orders of magnitude as shown, recently, by means of FETAX (Bernardini et al., 1994) and, in this paper, by Microtox (Table 1).

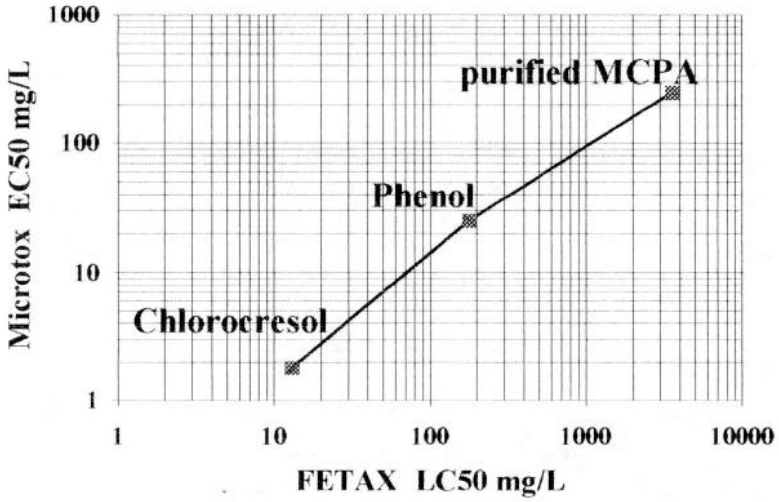

Figure I. Median effective concentrations obtained with Microtox plotted versus median lethal concentrations obtained with FETAX (Bernardini *et al.*, 1994).

The correlation between Microtox and FETAX results is shown in Fig. 1 by plotting the EC_{50s} values obtained with Microtox test versus the L C_{50s} obtained by FETAX; in this case Microtox is certainly more sensitive and less time consuming than FETAX. FETAX, however, is capable of detecting teratogenesis; in addition, the most sensitive FETAX endpoint, the MCGI (Minimun Concentration that determines Growth Inhibition) is comparable with the Microtox EC_{50} (Table 3).

In conclusion, an evaluation of the toxic effects of a chemical can only be given if the purity of the substance itself as well as the toxicity of impurities and additives has been taken into account. In fact, commercial compounds, generally, contain impurities and, if formulated, excipients; in this case toxicological tests confirm the synergistic effects of the different chemicals present in the compound that actually pollute the environment.

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