Sensitivity of the algal biotest ISO 10253 to the photosystem 2 herbicides in seawater

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Abstract

The sensitivity of marine algal biotest ISO 10253 to the photosystem 2 (PS2) herbicide diuron (DCMU) was determined. Using the diatom Phaeodactylum tricornutum, we found that the algal growth rate was reduced to 50 % of the control value (EC₅₀) for ca. 200 nM DCMU. This value is too high to allow a practical application of the biotest for concentrations of the PS2 herbicides found in natural waters. The mechanisms causing the low sensitivity of the biotest to the PS2 herbicide were investigated by measuring parameters of photosynthetic apparatus in the diatom prior and during the biotest. The apparent dissociation constant for DCMU in P. tricornutum found by measurements of inhibition of oxygen evolution and of variable fluorescence was in the range 60-90 nM. This should lead to a much higher sensitivity of the biotest than found in our experiments. The low biotest sensitivity is caused by an acclimation to sub-lethal DCMU concentrations. The acclimation is manifested by the chlorophyll content per cell that is increasing with the DCMU concentration. During a prolonged exposure to sub-lethal herbicide concentrations, we observed also a selection of DCMU resistant organisms indicating that also an adaptation may decrease the test sensitivity. The biotest sensitivity may

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Abbreviations: Chl - total chlorophyll (a+c₁+c₂); DCMU - 3-[3,4-dichlorophenyl]-1,1-dimethylurea; EC₅₀ - effective concentration of the substance resulting in a 50 % reduction in the growth rate; Kₐₕₕ - apparent dissociation constant; PS - photosystem; Qₐ and Qₐₕ - the primary (A) and the secondary (B) quinone acceptors of photosystem 2; RC - photosystem 2 reaction centre.

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increase when the acclimation and adaptation are limited by shortening of the experiment duration.

Additional key words: bioassay; chlorophyll fluorescence; DCMU; 3-[3,4-dichlorophenyl]-1,1-dimethyleurea; diuron; *Phaeodactylum tricornutum*; quinone acceptors.

**Introduction**

Herbicides acting on PS2 are widely used for an inexpensive elimination of weeds in agriculture. Some of the PS2 herbicides, most notably 3-(3,4-dichlorophenyl)-1,1-dimethyleurea, have been also extensively used as a principal tool of photosynthesis research (see Duysens and Sweers 1963 and Izawa 1977 for reviews) and the molecular mechanism of their action on plants is known. The herbicide molecule binds in the Q$_B$-binding pocket of the D1-protein of PS2 where otherwise plastoquinone molecules accept electrons from the primary quinone acceptor Q$_A$ (e.g., Duysens and Sweers 1963, Trebst and Draber 1986, Draber *et al.* 1991, Krause and Weis 1991). Thus, the herbicide binding blocks the electron transport from the PS2 to the cytochrome b/f complex and prevents the photosynthetic energy conversion.

A fraction of the herbicides applied in agriculture is washed into natural waters and represents a serious pollution problem. Herbicides can be toxic to human and animal health (Stevens and Sumner 1991) and application of some of the herbicides has been restricted by law in the most developed countries (Ware 1986). Yet, the peak concentrations of some restricted-use PS2 herbicides (*e.g.*, atrazine) that are found in surface waters may reach tens or even hundreds in nanomolar concentrations (Huber 1993, Bintein and Devillers 1996, Solomon *et al.* 1996). Typical values are much lower and sensitive gas and high-pressure liquid chromatography have to be applied on samples pre-concentrated either by extraction or in cartridges (Bardalaye and Wheeler 1986, Wells *et al.* 1994, Lacorte *et al.* 1998).

The sensitivity of the algal growth inhibition biotests is not sufficient to detect these levels of the herbicide pollution (El Jay *et al.* 1997) although the corresponding acute inhibition of the photosynthetic reactions may be substantial. Here, we investigate and compare the results of the biotest with measurements of the acute inhibition of photosynthesis in order to identify mechanisms that limit the biotest sensitivity.

**Materials and methods**

**Marine algal growth inhibition test ISO 10253:** Sterile artificial sea water prepared according to ISO 10253 (100 cm$^3$ in 250 cm$^3$ glass Erlenmeyer flasks) was poisoned with DCMU (*Riedel-de Haën*, Seelze, Germany) and inoculated by *Phaeodactylum tricornutum* Bohlin strain Cough (CCMP, Bigelow Laboratory for Ocean Sciences, W. Boothbay Harbor, Maine 04575, USA) to the final concentration of 10 000 cells per cm$^3$. Two flasks were used for each herbicide concentration. The herbicide was