

## PHOTOMODIFICATION EFFECT ON THE PHOTOINDUCED TOXICITY OF FOURTEEN ANTHRAQUINONES TO *DAPHNIA MAGNA*

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### Abstract

The toxicity of chemicals can be enhanced by light through two pathways: photomodification to more toxic substances and photosensitization reactions. The photoinduced toxicity of polycyclic aromatic hydrocarbons (PAHs) has been well documented; however, little information is available concerning other environmental contaminants. Anthraquinones derivatives (AQs) are a group of important chemicals used in dye, pharmaceutical and pulp industries. The photoinduced toxicity of AQs potentially exists due to their absorption of visible and ultraviolet light. Moreover, some AQs can be phototransformed to other chemicals. The photomodification effect on the photoinduced toxicity of AQ dyes to *Daphnia magna* was assessed in the present study. Ten of 14 studied AQs showed phototoxic activity under simulated solar radiation (SSR). The photoinduced toxicity of six unphotostable AQs was reduced when they were photomodified under SSR. The order of medium effective concentration ( $EC_{50}$ ) ratio (photomodified/intact AQs) was almost consistent with the rate of photomodification. The present study can provide useful information for the ecological risk of AQs and a better understanding of their potential photoinduced toxicity mechanism.

### Introduction

Some organic chemicals such as polycyclic aromatic compounds exert photoinduced toxicity to numerous aquatic species in the presence of solar radiation<sup>1,2</sup>. Anthraquinones derivatives (AQs) are commonly used in the textile industry. Nearly 12% of the dyes used yearly are lost during dyestuff manufacturing and textile dyeing operations, and 20% of these losses are released to the environment via effluents. Thus, the photoinduced toxicity of AQ dyes to aquatic organisms needs to be focused on due to their light absorbance property.

It is believed that the mechanism of photoinduced toxicity can be generally classified as photosensitization associated with generation of reactive oxygen species (ROS) and photomodification to more toxic photoproducts. Photomodification of several polycyclic aromatic hydrocarbons (PAHs) was capable of producing more reactive and biological damaging photoproducts<sup>3-5</sup>. Photochemical transformation of some AQs can occur in aqueous or organic solution. Thus, it is necessary to investigate the variation of the photoinduced toxicity of AQs in the presence of the solar light.

The objective of this study was to examine the effects of photomodification on the photoinduced toxicity of AQs. In our previous study, the acute toxicity of 14 AQs to *Daphnia magna* were determined under simulated solar radiation (SSR, visible light plus UV-A and UV-B)<sup>6,7</sup>. To assess the influence of photomodification, we exposed

*D. magna* to photomodified AQs under SSR to examine the variation of photoinduced toxicity compared with the intact AQs.

### Materials and Methods

Fourteen AQs with substituents -NH<sub>2</sub>, -Cl, -Br, -NO<sub>2</sub>, -SO<sub>3</sub>H or -OH were obtained from the State Key Laboratory of Fine Chemicals (Dalian University of Technology) and Sinochem Liaoning Imp. & Exp. Corp. They were dissolved in dimethyl sulfoxide (DMSO) to make a primary stock solution. The final concentration of DMSO in the test solution was a constant of 0.1%.

*D. magna* were cultured under cool-white fluorescent light with a 14-h light/10-h dark photoperiod. *Scenedesmus obliquus* were fed to *D. magna* daily. The acute toxicity assay with neonates (< 24 h old) of *D. magna* was performed in all bioassays. Medium effective concentration ( $EC_{50}$ ) values with 95% confidence limits were calculated using Probit analysis (US EPA, 1993). Values presented as means  $\pm$  standard deviation (SD). Radiation sources (SSR) for toxicity assays and photostability experiments included a series of fluorescent tubes.

To investigate the photostability, AQ solutions were placed inside of Pyrex tubes under SSR or in the dark for 48 h. The sample solutions except water soluble 1-amino-4-bromoanthraquinone-2-sulfonic acid were extracted three times with CH<sub>2</sub>Cl<sub>2</sub> for chemical analysis. The analysis was carried out using an Agilent 1100 HPLC equipped with a diode array detector (DAD).

AQ solutions of different concentrations were pre-exposed to SSR or kept in the dark for 48 h. Then daphnids were transferred to test the acute toxicity of the solutions under SSR and their immobilization was recorded after 24 h. All the toxicity and photolysis experiments were conducted on a handmade merry-go-round apparatus.

### Results and Discussion

In the dark, 14 tested AQs exhibited no observable toxicity in the maximum test concentration available. Three nitro-substituted AQs and 1,2-dihydroxyanthraquinone were non-phototoxic under SSR. The other 10 AQs showed a toxicity increase under SSR with a range of  $EC_{50(\text{dark})}/EC_{50(\text{SSR})}$  from 16.8 to 2083.3.

The photoinduced toxicity of AQs together with their photoproducts formed by SSR was investigated under SSR to evaluate the photomodification effect. The control experiments showed the loss of all the tested AQs in culture medium was negligible in 48 h by HPLC analysis. Four of 10 phototoxic AQs including 1,8-dichloroanthraquinone, 1,5-diaminoanthraquinone, 1-amino-4-bromoanthraquinone and 1-amino-4-bromoanthraquinone-2-sulfonic acid were photostable under SSR.

The photomodification half-lives of the other six AQs in culture medium ranged from 9.3 h to 72.2 h. Then we selected six phototoxic AQs to evaluate the photomodification effect on the photoinduced toxicity to *D. magna* under SSR. In general, photomodification can decrease the photoinduced toxicity of the six AQs to *D. magna* under SSR. There may be less toxic substances formed for them in the aqueous environment under SSR. It

should be noted that the order of  $EC_{50}$  ratio (photomodified/intact) was nearly coincident with the photomodification rate. Significant correlation relationship was observed between the order of  $EC_{50}$  ratio and the photomodification rate ( $R = 0.952$ ,  $p < 0.01$ ), suggesting that the photoinduced toxicity of the photomodified AQs with more rapid photomodification rate decreased more dramatically. 1,8-dihydroxyanthraquinone had the fastest photomodification rate ( $k_{obs} = 0.0746 \text{ h}^{-1}$ ) and remained only 2.3 % of the intact compound after 48 h photomodification. The rapid photomodification can result in the toxicity decrease of photomodified 1,8-dihydroxyanthraquinone by a factor of greater than 19 compared with the intact chemical.

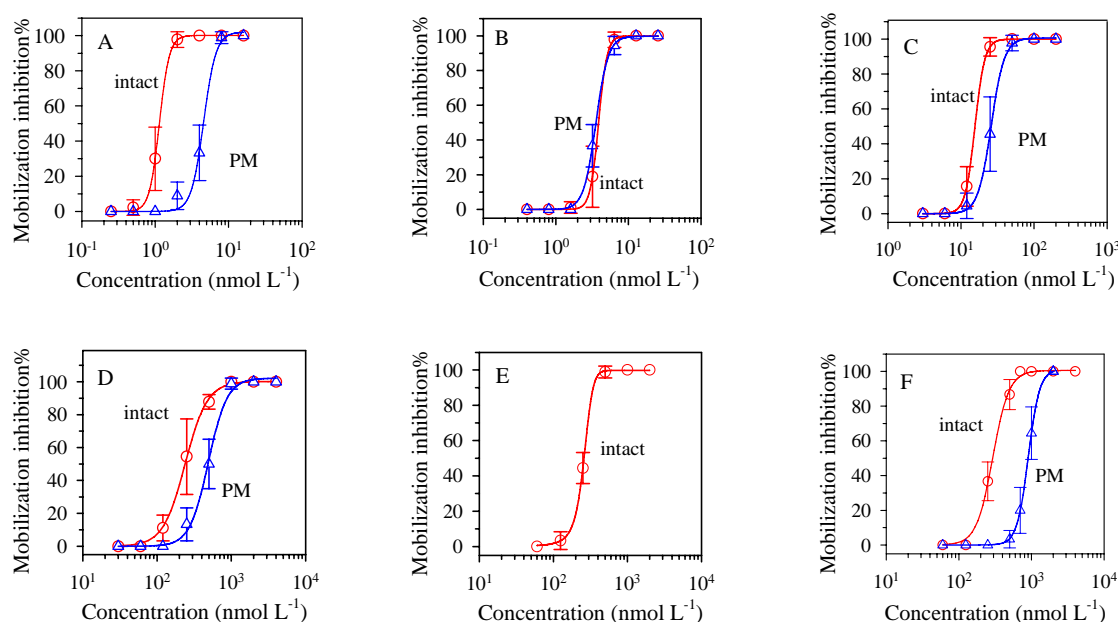


Figure 1. Concentration-response curves for six intact and photomodified (PM) AQs including (A) 1-amino-2,4-dibromoanthraquinone, (B) 1-amino-2-methyl-4-bromoanthraquinone, (C) 1-amino-2-bromoanthraquinone, (D) 1-chloroanthraquinone, (E) 1,8-dihydroxyanthraquinone and (F) 1-aminoanthraquinone to *D. magna* under simulated solar radiation

Clear concentration-response relationships were observed for the photomodified AQs and intact AQs to *D. magna* under SSR (Fig. 1). Three photomodified AQs (1-amino-2,4-dibromoanthraquinone, 1-chloroanthraquinone and 1-amino-2-bromoanthraquinone) demonstrated dramatically decreased toxicity under SSR compared with the intact chemicals. There was a clear trend in the slope of the concentration-response curves for the intact AQs with the amino substituent. The slope increased when introducing the Br atom based on the 1-aminoanthraquinone. Additionally, three amino- and bromo-substituted AQs including 1-amino-2,4-dibromoanthraquinone, 1-amino-2-bromoanthraquinone and 1-amino-2-methyl-4-bromoanthraquinone had the approximate slope, which may result from their same modes of action.

Several AQs including 1,8-dihydroxyanthraquinone and 1,2-dihydroxyanthraquinone were more toxic to the duckweed after photomodification<sup>8</sup>. For PAHs, the photomodification can increase the photoinduced toxicity of the parents to some aquatic species<sup>9</sup>. However, Choi and Oris found that the photomodified products (anthraquinone, 1,2-dihydroanthraquinone) of anthracene reduced the parent photoinduced toxicity based on *in vitro* toxicity assays using a fish hepatoma cell line<sup>10</sup>. In our previous study, photomodification can decrease the photoinduced toxicity of 1-amino-2,4-dibromoanthraquinone to *D. magna* perhaps due to the generation of more hydrophobic substances<sup>3</sup>. This indicated that the impacts of the photomodification on the photoinduced toxicity may depend on the physicochemical properties of the photoproducts and the toxicological endpoint.

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