

The Effects of Binary Combinations of Cadmium, Carbendazim and Ultraviolet Radiation on *Daphnia magna*

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(Received 25 January 2010; accepted 16 February 2010)

Abstract—Nowadays risk assessment procedures have focused on effects of single toxic compounds and controlled conditions to evaluate or preview environmental contamination. However this prediction is not accurate because in their natural habitats, organisms are exposed to more than one single chemical and additionally to changes in natural conditions (e.g. temperature, radiation). Increasing amount of solar UV radiation reaching water bodies causes, by itself, harmful effects on zooplankton, and it can also lead to a photo-enhanced toxicity of chemicals that are present in these systems. Therefore, in order to evaluate single and combined toxicity of cadmium and carbendazim under ultraviolet radiation to *Daphnia magna*, we performed acute and chronic bioassays, in accordance with OECD guideline 202 and 211 respectively. The conceptual model of Independent Action (IA) and possible deviations for synergism, antagonism and dose ratio or dose level dependencies were used to derive response patterns of these combined exposures. For survival and reproduction endpoints, neither cadmium nor carbendazim showed to have a photo-activated toxicity by the presence of ultraviolet radiation. Moreover, it was observed an antagonism response pattern for the combination between ultraviolet radiation and carbendazim.

Keywords: *Daphnia magna*, cadmium, carbendazim, ultraviolet radiation, independent action

INTRODUCTION

The natural conditions of the environment are not always present in their optimal range for organisms. Therefore most species are likely to face a combination of stress factors such as mixtures of different environmental pollutants and/or an interaction between a natural and a chemical stressor (Holmstrup *et al.*, in press). The ultraviolet radiation (UVR) has been considered a threat to earth's life since the early 70's when the thinning of the ozone layer started being reported, reaching a decrease of 3% since 1980 (IPCC, 2005). Afterwards a variety of studies have proved the deleterious effects of increasing intensities of ultraviolet radiation on organisms both aquatic and terrestrial (Häder *et al.*, 2003; Caldwell *et al.*, 2007). Moreover the presence of environmental pollutants creates scenarios

of combined exposures to natural stressor (e.g. ultraviolet radiation) and chemical compounds. The aim of the present work was to evaluate the toxicity of cadmium and carbendazim under the presence of ultraviolet radiation. *Daphnia magna* was chosen to perform this study because of its representability in most freshwater ecosystem and for being an important key species in ecotoxicological assessments. The conceptual mode of Independent Action (IA) was used to derive the response patterns of endpoints such as immobilisation, feeding activity and reproduction.

MATERIALS AND METHODS

Combination of chemicals and ultraviolet radiation were carried out and simultaneously single exposure for both stressors were also evaluated.

Immobilisation

Immobilisation approach was performed according to OECD 202 (OECD, 2004) by exposing animals to ultraviolet radiation in contaminated ASTM (ASTM, 1980) medium with cadmium and carbendazim. Nominal concentrations ranged from 25 to 250 $\mu\text{g/L}$ for cadmium and 80 to 200 $\mu\text{g/L}$ for carbendazim. Ultraviolet radiation intensities were achieved by varying times of exposure to the UV lamp and ranged from 105 to 450 $\text{J}\cdot\text{m}^{-2}$.

Feeding inhibition

Neonates at the age of 4d-old were used. The proceeding for feeding inhibition bioassay was adapted from McWilliam and Baird (2001) and divided into three phases: a) Combined exposure period to UV-radiation and chemical contamination; b) Feeding period: after irradiation time, daphnids were allowed to feed for 24 h in a contaminated medium at the same concentration that was used in the exposure period. *Pseudokirchneriella subcapitata* was provided as food source. c) Post-exposure period: after feeding period, daphnids were transferred to a "clean" medium (i.e. no chemical contamination) and allowed to feed for 4-hours. Following feeding and post-exposure period, absorbance measurements of the medium were taken. Nominal concentrations ranged from 10 $\mu\text{g/L}$ to 50 $\mu\text{g/L}$ for cadmium and 12.5 $\mu\text{g/L}$ to 100 $\mu\text{g/L}$ for carbendazim.

Reproduction

Reproduction bioassay was adapted from OECD 211 guideline (OECD, 1998) for both cadmium and carbendazim. When reached the age of 6d old (where the egg formation takes place) daphnids were exposed to UV-radiation in ASTM medium. Individuals from each concentration were exposed together to the UVR which corresponds to one daphnia/time of exposure/concentration. UV-control treatment (ASTM without chemical contamination) was performed. After exposure period, daphnids were transferred to contaminated medium at the same concentration applied in the exposure to ultraviolet radiation. Daphnids were placed individually in 50 ml glass vials inside a controlled chamber until complete 21d-old. Neonates produced along the assay were counted in each

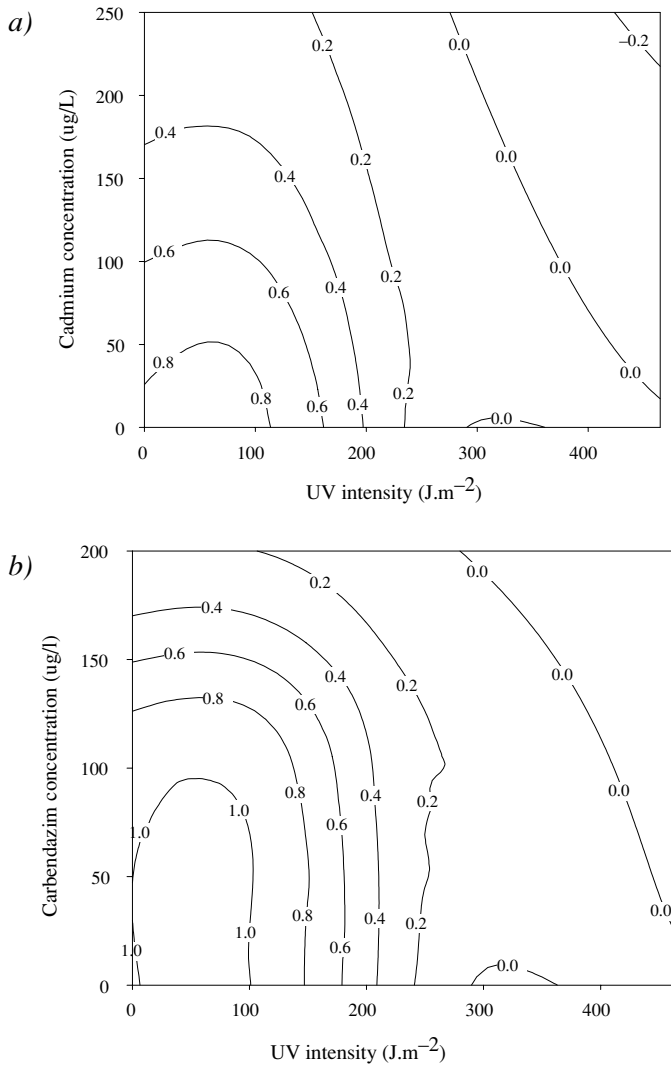


Fig. 1. a) Dose-response relationship for the combination of cadmium and ultraviolet radiation to the survival of *D. magna*, showing an adjustment to the IA model. b) Dose-response relationship for the combination of carbendazim and ultraviolet radiation to the survival of *D. magna*, showing an adjustment to the IA model.

treatment and test-medium was renewed every other day. Nominal concentrations ranged from 12.5 $\mu\text{g/L}$ to 75 $\mu\text{g/L}$ for carbendazim and from 0.05 $\mu\text{g/L}$ to 2.0 $\mu\text{g/L}$ for cadmium. To calculate the joint toxicity of chemical and ultraviolet radiation exposures, the response profile for UVR was first described, and the information was used to calculate experimental design for combinations. The

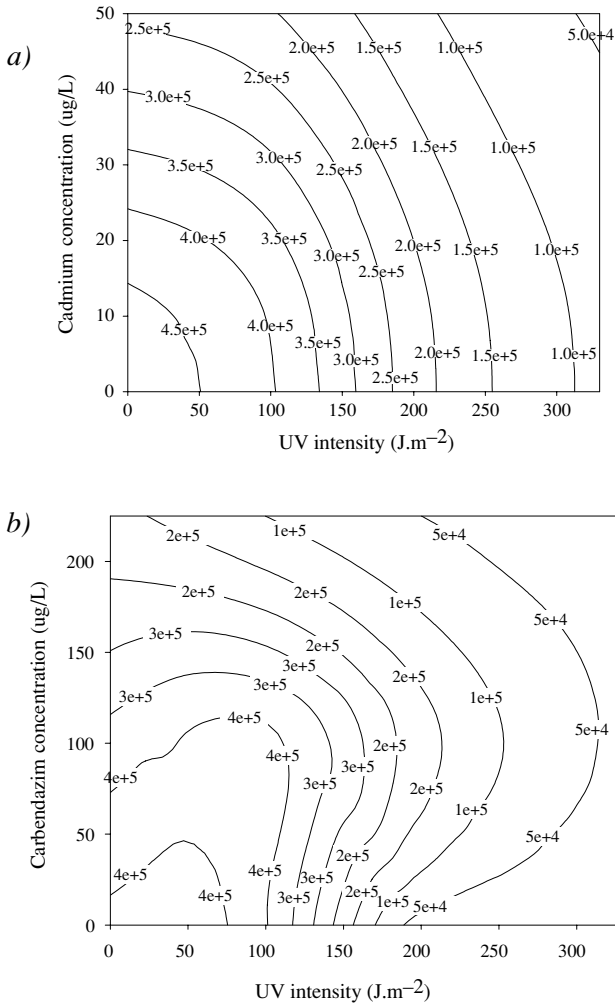


Fig. 2. a) Dose-response pattern for the combination of cadmium and ultraviolet radiation to the feeding activity of *D. magna*, showing an adjustment to the IA model. b) Dose-response pattern for the combination of cadmium and ultraviolet radiation to the feeding activity of *D. magna*, showing a dose-ratio deviation from the IA model.

expected effect of combinations was based on the dose-response curve for each of the components and calculated with the MixTox model (Jonker *et al.*, 2005) that predicts biological response for both chemical mixtures and combinations of chemical and natural stressor, using the reference model of Independent Action (IA) as well as the deviations from the model “S/A”, “DL” and “DR” dependence, which were obtained with by the addition of parameters *a* and *b* to the equation.

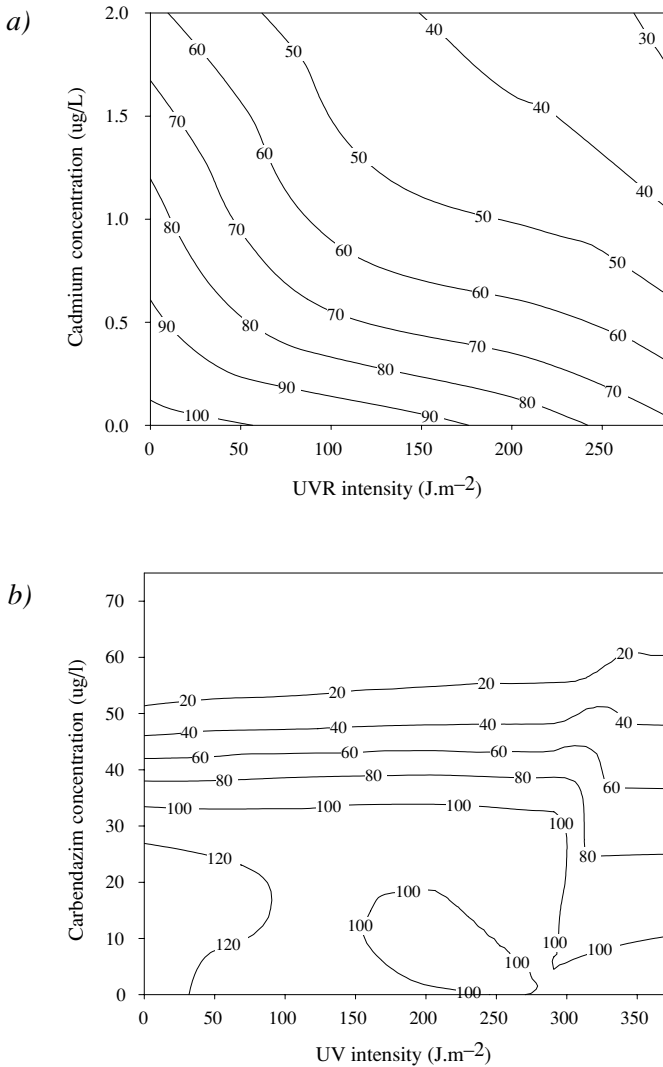


Fig. 3. a) Dose-reponse pattern for the combination of cadmium and ultraviolet radiation to the reproduction response of *D. magna*, showing a synergism deviation from the IA model. b) Dose-reponse relationship for the combination of carbendazim and ultraviolet radiation to the reproduction of *D. magna*, showing a dose-ratio deviation from the IA model.

RESULTS AND DISCUSSION

Data from acute exposure of *D. magna* to cadmium and UVR was best fitted to the IA model, with no significant deviation to synergism or antagonism ($R^2 = 0.77, p < 0.05$) (Fig. 1a). For feeding activity endpoint, data was also fitted to the IA model ($R^2 = 0.84, p < 0.05$), showing also no significant deviations (Fig. 2a).

Ferreira *et al.* (2008) found the same pattern for the feeding response of *D. magna* to the combination of cadmium and dissolved oxygen when fitted to the IA model. For the reproduction response, data was well described by the IA model ($R^2 = 0.60$, $SS = 6875.42$, $p < 0.05$). Adding parameter a to the equation, the residual value was decreased and the R^2 was increased, showing significant adjustment to synergism ($a = -2.3$, $R^2 = 0.64$, $SS = 6080.81$, $p < 0.05$) and supported by the decrease of the EC_{50} of cadmium with increasing ultraviolet radiation doses (Fig. 3a).

For the combination of carbendazim and ultraviolet radiation, survival data fitted significantly to the IA model ($SS = 13.57$, $r^2 = 0.91$, $p < 0.05$) (Fig. 1b). No deviations from the model meant that the carbendazim and UVR had no relations and/or competition for target sites in the organism at lethal doses. For feeding inhibition exposure, the IA model fitted also our data ($SS = 9.7 \times 10^{-10}$, $R^2 = 0.85$, $p = 1.96 \times 10^{-15}$). Adding parameter a to the equation, we observed a significant adjustment to antagonism ($SS = 7.81 \times 10^{-10}$, $a = 1.90$, $p = 0.002$). The dose-ratio deviation from S/A was also tested by adding parameter b and was the best description for the data ($R^2 = 0.93$, $SS = 3.34 \times 10^{-10}$, $p = 3.04 \times 10^{-8}$) (Fig. 2b). The antagonistic toxicity of this combination was mainly caused by the UV radiation as indicated by the interpretation of the additional parameters a and b ($a = -10.35$, $b = 23.69$). The decreased in feeding activity of *Daphnia magna* was mainly caused by the ultraviolet radiation. Lacuna and Uye (2000) found impairments on feeding rates of the copepod *Sinocalanus tenellus* after exposure to UV radiation. Regarding the reproduction response for this exposure, a good adjustment of data set to the IA model was observed ($SS = 2929.2$, $r^2 = 0.95$, $p = 1.29 \times 10^{-13}$). By adding parameters a and b_{DR} , a Dose-Ratio deviation was concluded ($a = 27.58$ and $b = -450.8$) (Fig. 3b). By the interpretation of values of a and b (Jonker *et al.*, 2005) this case shows synergism when the toxicity of the combination was mainly caused by the UV radiation.

Acknowledgments—The study was partly supported by the EU Integrated project NoMiracle (Novel Methods for Integrated Risk assessment of Cumulative Stressors in Europe; <http://nomiracle.jrc.it>) contract No. 003956 under the theme under the EU-theme “Global Change and Ecosystems” topic “Development of risk assessment methodologies”, coordinated by Dr. Hans Løkke at NERI, DK-8600 Silkeborg, Denmark, and partly financed by the FCT-Fundação para a Ciência e Tecnologia project “Assessing the combined effects of chemical stressors and UV radiation on *Daphnia magna*” (PTDC/AMB/74346/2006), coordinated by Prof. Dr. Amadeu M. V. M. Soares.

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