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Ultraviolet Radiation Increases Sensitivity to Pesticides: Synergistic Effects on Population Growth Rate of *Daphnia magna* at Low Concentrations

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Abstract In the present study we aimed to investigate whether UV-B radiation can exacerbate effects of pesticides fenoxycarb, pirimicarb, and tebufenpyrad on the survival, reproduction, and population growth rate of the standard test species *Daphnia magna*. We applied sublethal pesticides' concentrations and UV doses and observed no effects on survival. However, we observed synergistic effects of UV and pesticides on both cumulative reproduction and population growth rate (21 days) for fenoxycarb (100 μg/L) and pirimicarb (10 μg/L), but a less-than-additive effect for tebufenpyrad (5–10 μg/L). In the series exposed to UV and fenoxycarb or pirimicarb, the population growth rate dropped down to 0.1, while in the control series it was around 0.3. The results indicate that concentrations of some toxicants that are nontoxic in standard tests can cause harmful population-level effects when combined with UV.

Keywords *Daphnia magna* · Pesticide risk assessment · Standard toxicity test · UV

Despite considerable progress, ecological risk assessment of pesticides and other toxicants remains uncertain. The concentration levels of toxicants that either cause biological effects or can be defined as safe vary between different study systems, levels of biological organisation, and environmental conditions. Thus, when the effective concentration levels were compared among laboratory, mesocosm, and field studies recently, considerable differences were detected between the study systems (Beketov et al. 2008). There is evidence that, in the field, pesticides can have effects at concentrations more than 100 times below the acute median effective concentration derived for the standard test species *Daphnia magna* (EC50, the concentration that cause immobilization of 50% of the organisms) under laboratory conditions (e.g. Liess and von der Ohe 2005). In meso- and micro-cosm studies, the effective concentration varies to a comparable extent depending on the community characteristics, stress exerted on the organisms, and environmental conditions (Liess and Beketov 2011; Stampfli et al. 2011).

One reason for these differences is that organisms in real-world ecosystems are exposed to multitudes of biotic and abiotic stressors that are present in the environment but absent in standard toxicity tests. For example, recently, Rohr et al. (2008) showed that concentrations of toxicants that were shown previously to be nontoxic in laboratory tests can cause harmful large-scale effects on amphibians when combined with parasitic infections in a real-world environment. Similarly, when additional stressors are included in smaller-scale laboratory test systems, toxicants can cause effects at low concentrations that are comparable to those observed in the field. These additional stressors can be abiotic (e.g. Heugens et al. 2001), biotic (e.g. Beketov and Liess 2006) or a combination of the two (Liess et al. 2001).

Climate change and global changes in general have highlighted the need for improved consideration of environmental factors as additional stressors in ecotoxicological evaluations. One of the ubiquitous and biologically relevant abiotic factors whose importance has increased in the last two decades is solar ultraviolet (UV) radiation. Exposure of the surface of the earth to UV radiation has

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increased dramatically since the 1980s, mainly due to ozone depletion, and, despite certain improvements, has not reached the initial level (WMO 2007). Furthermore, at some mid-latitude locations in the northern hemisphere, surface UV irradiance continues to increase. This trend can be explained by optical effects of aerosols in the air, air pollution, and partly by a decreased amount of clouds (WMO 2007).

Detrimental effects of UV are well known for all the major taxonomic groups from bacteria to higher plants and vertebrate animals in both terrestrial and aquatic habitats (Young et al. 1993). The component of UV that is most dangerous to biological organisms is the short-wave UV-B radiation (280–320 nm), which has been shown to cause DNA damage, inhibit photosynthesis, affect locomotion, suppress reproduction, reduce body size, and increase mortality (Preston et al. 1999; Williamson et al. 2001a, 2002). Furthermore, it was shown that UV might also cause large-scale effects on community structure of lake zooplankton (Williamson et al. 2001b), as well as result in combined effects with other factors (Liess et al. 2001; Williamson et al. 2002).

It might be expected that UV radiation and environmental toxicants have combined additive or even synergistic effects as ubiquitous and relevant stressors. However, the large majority of investigations on UV and toxicants have addressed chemical interactions between these two factors, but not their combined biological and ecological effects. So-called photoactivation of toxicants, namely, chemical changes that are caused by UV and the resulting increases in toxicity, has been shown repeatedly for polycyclic aromatic hydrocarbons (e.g. Huovinen et al. 2001). Similar effects were also found recently for sulfonamide antibiotics (Jung et al. 2008). To the best of our knowledge, combined biological effects of UV and environmental toxicants (i.e. without photoactivation) have only been shown in three studies on metals (Preston et al. 1999; Liess et al. 2001; Duquesne and Liess 2003) and one study on pentachlorophenate sodium salt (Preston et al. 1999).

With regard to pesticides, neither photoactivation-based increases in toxicity nor the combined biological effects of these toxicants and UV have been investigated. However, given the wide importance of UV radiation, it is possible that UV and pesticides have additive or synergistic effects, and that these interactions can explain at least partially the differences between the effects of certain concentration levels in the field and in standard laboratory toxicity tests. Furthermore, knowledge about such interactive effects is essential to predict the consequences of ozone depletion and increased UV irradiance, as well as to determine the concentrations of toxicants that are safe under the conditions of climate change.

The aim of the study was to understand whether UV-B radiation and exposure to three selected pesticides at sublethal doses/concentrations that could be encountered realistically in the environment can have combined additive or synergistic effects on the survival, reproduction, and particularly population growth rate (r) of the standard test species *D. magna* (OECD 1997).

Materials and Methods

Standard 21-day chronic reproduction tests with *D. magna* (OECD 1997) were conducted in the presence and absence of UV-B radiation. To exclude the effect of the UV on the chemical structure of the toxicants, we exposed the daphnids to the pesticides and UV at separate times. Specifically, we exposed newborn neonates (<24 h old) to pesticides for 24 h at the start of the test to mimic field-relevant pulse exposure (Liess et al. 1999; Beketov and Liess 2008a). After the pesticide exposure and subsequent rinsing, the daphnids were maintained in clean medium and exposed daily to UV radiation. This approach could not exclude the possibility of UV-triggered chemical transformation (i.e. photoactivation) of pesticide that was retained inside the bodies of the tested daphnids after the initial exposure, but could exclude the possibility of photoactivation in the solutions tested. Essentially, this system represents realistic short-term exposure to pesticide and long-term exposure to UV (it also reflects the subsequent exposure of the organisms to these two stressors, as pesticides mainly occur in fresh waters due to surface runoff following strong rainfalls when UV irradiation is reduced).

In each test, 28 neonates aged less than 24-h old were used for each concentration of the three pesticides described below. Each neonate was exposed individually in a 100-mL beaker that contained 50 mL of test solution. In addition, 40 individuals in a control series were exposed to clean M7 medium (OECD 1997). The organisms were not fed during the exposure. After 24 h of exposure, all the animals were rinsed and transferred to 50 mL of fresh M7 medium. Following this procedure, the beakers were distributed randomly between the two setups: one group was exposed to normal light, which was provided by fluorescent tube lamps, and the other was exposed to UV-B light (intensity around 0.11 mW/cm2) for 4 h per day in addition to the normal light. The fluorescent lamps were Sylvania Luxline plus, F36 W/840 Cool White De Luxe (Haarlem, Netherlands), and Osram L 36 W/840 Cool White (Munich, Germany). The colour temperature of the both lamp types is 4,000 K and the nominal luminous flux at 25°C is 3,300 Lm. These lamps were selected, as they have been used routinely for the standard *D. magna* culture. The UV lamps were Vilber-Lourmat, T40 M (Vilber–Lourmat

Deutschland GmbH, Eberhardzell, Deutschland). The UV spectrum (Fig. 1) included two peaks: the highest peak at 312 nm (UVB range) and the lower one at 363 nm (UVA range) (data from the manufacturer, Vilber–Lourmat Deutschland GmbH). Therefore, the spectrum included not only the damaging UVB, but also photorepairing UVA (Williamson et al. 2001a). The intensity of the UV-B light was selected to represent exposure on a sunny day in the region of the study (on the basis of our measurements in the area of the research centre), and was lower than the intensity reported for more southerly latitudes (e.g. up to 0.2 mW/cm^2 in Korea, Jung et al. 2008). The selected intensity was also confirmed not to cause mortality of the daphnids in preliminary tests. Both setups were maintained under identical conditions in ventilated metal test chambers with a temperature of 20°C and a 16/8 h light/dark photoperiod. The daphnids were fed and the medium changed three times per week, at which times survival was also checked and offspring counted. A detailed description of the culture maintenance and test conditions for *D. magna* can be found elsewhere (Beketov and Liess 2008b).

The three pesticides selected for the experiments were fenoxycarb, pirimicarb, and tebufenpyrad. Both fenoxycarb and pirimicarb belong to the carbamate class, but they have different modes of action. Fenoxycarb mimics the action of juvenile hormone, acting as a specific growth regulator, whereas pirimicarb is an acetylcholinesterase inhibitor. Tebufenpyrad is a pyrazolic acaricide that blocks mitochondrial electron transport. All three compounds are approved for use in most EU countries and the USA.

The acute (48 h) median effective concentrations (EC50) of fenoxycarb, pirimicarb, and tebufenpyrad for

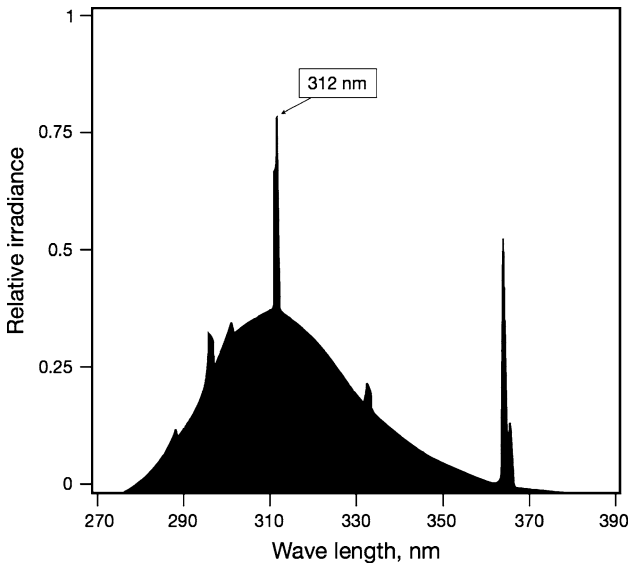

Fig. 1 The spectrum of the UV lamps (the major peak at 312 nm is noted)

D. magna described in the literature are 500, 16–24, and 46 μg/L, respectively (Andersen et al. 2006; http://sitem.herts.ac.uk/aeru/footprint/en/). Given that the duration of exposure in our 21-days tests was shorter than that in these published studies (24 instead of 48 h), we performed preliminary tests with a 24-h exposure period and a subsequent 72-h observation period (a total of 96 h). In these tests, we applied six concentrations, which included a control, and used seven replicates for each concentration (one neonate per each replicate). The median lethal concentrations (LC50, the concentration that causes mortality of 50% of the organisms) derived in these preliminary tests were higher than EC50s known from literature, obviously due to a much shorter exposure period (Table 1, we compare here LC50 s and EC50 s as identical endpoints, as after 96 h in our tests immobilisation recorded for EC50 s was equal to death).

The concentrations applied in the 21-days tests were 5 and 10 μg/L for both pirimicarb and tebufenpyrad, and 100 μg/L for fenoxycarb. These concentrations were chosen to be well below the acute LC50 s (Table 1) and to be field-relevant for pirimicarb and tebufenpyrad (Beketov and Liess 2008c). The fenoxycarb concentration was higher than the usual field-relevant range, but it was selected to have a similar range of differences from its respective acute LC50 as in the case of the other two test compounds (Table 1). To ensure the quality of the solutions applied in the 21-days reproduction tests, the test solution for one of the three pesticides, tebufenpyrad, was analysed by gas chromatography (analysis was performed by Kommunale Wasserwerke Leipzig GmbH, Leipzig, Germany). This analysis was performed using the 100 μg/L (nominal concentration) tebufenpyrad solution and the concentration measured was 96 μg/L, which confirmed the precision of the solution preparation. Although the other compounds were not measured, we do not expect considerable differences between the nominal and real concentrations, as confirmed by measurements from previous experiments with the same substances.

The LC50 values were calculated by the Trimmed Spearman–Karber method (Hamilton et al. 1977) using the program Spearman (Montana State University, Bozeman, MT, USA). This method is a nonparametric statistical procedure that is effective for different concentration–response curve shapes including non-monotonic results. The population growth rate (r) was calculated on the basis of age-specific data on survival and fecundity probabilities. The iterative calculation was performed using the Lotka/Euler equation (Lotka 1913; Euler 1970; see also Forbes and Calow 1999). The variance associated with r-values for statistical comparison was estimated by the jackknife method in accordance with Meyer et al. (1986). Statistical comparisons were performed using the non-parametric

Table 1 Median lethal concentrations (LC50) of fenoxycarb, pirimicarb, and tebufenpyrad for *Daphnia magna* in the tests with 24-h exposure and subsequent 72-h observation (μg/L, 95% CI in parentheses)

Pesticides	Median lethal concentrations (LC50) and 95% CI, μg/L
Fenoxycarb	1,831.2 (1190.4–2817.0)
Pirimicarb	29.08 (17.54–48.21)
Tebufenpyrad	67.94 (39.99–115.44)

Kruskal–Wallis test followed by a non-parametric multiple comparison test of the Behrens–Fischer type (Munzel and Hothorn 2001). The non-parametric methods were applied because the data failed to demonstrate a normal distribution and homogeneity of variances. A common two-way analysis of variance approach was not applied owing to non-linear concentration–response dependences. All statistical analyses and production of graphics were carried out using the software package R, version 2.10 for Mac OS X (http://www.r-project.org/).

Results and Discussion

The median lethal concentrations (LC50 s) derived from the preliminary tests are summarized in the Table 1. These LC50 values are higher than the EC50s described in the literature (Andersen et al. 2006; http://sitem.herts.ac.uk/aeru/footprint/en/) owing to the markedly shorter exposure period (we compare LC50s and EC50s as identical endpoints because, after 96 h in our tests, the rate of immobilisation recorded for EC50s was equal to the mortality rate).

As expected, no statistically significant mortality was detected for any of the treatments, which included UV and pesticides alone as well as in combination, as compared with the UV—and pesticide-free control, during the entire observation period ($p > 0.05$). Similarly, exposure to neither UV without pesticides nor pesticides without UV had any significant effects on the population growth rate (Fig. 2). However, this endpoint was affected significantly ($p < 0.05$) by the combination of UV with either fenoxycarb or pirimicarb at the highest concentrations tested (Fig. 2). The combination of UV and tebufenpyrad caused the largest decline in the population growth rate among all the treatments, but this effect was not significant (Fig. 2).

The cumulative rate of reproduction was not affected by any of the pesticides tested in the absence of UV, but exposure to UV alone caused a significant decrease in the number of neonates (Table 2). However, similarly to the case for population growth rate, reproduction was affected most strongly by the combinations of UV and the pesticides at the highest tested concentrations. Thus, for both fenoxycarb and pirimicarb, the numbers of neonates produced in these two-stressor series were significantly lower than those in any other series, which included the controls with UV (Table 2). With regard to tebufenpyrad, it also had a strong effect on reproduction when combined with UV, but this effect was not significantly different from the effect of UV alone (Table 2).

Effect size summation analysis showed that, for both population growth rate and cumulative reproduction, the combined effects of UV and the highest concentrations of fenoxycarb and pirimicarb were greater than the sums of their individual effects, namely, synergistic effects occurred (Fig. 2; Table 2). Other combinations of UV and pesticide showed effects that were less than the sum of their individual effects (namely, both concentrations of tebufenpyrad, and low concentrations of both fenoxycarb and pirimicarb).

As mentioned above, the combined effects of UV and pesticides have not been investigated previously. Therefore, this study represents a first step towards understanding the types of interaction that can occur between such stressors (e.g. synergistic or additive), and to a smaller degree, the extent to which UV can exacerbate the effects of pesticides on the non-target biota. The results of this study show that the combined effects of UV radiation and pesticides can be synergistic or less than additive, depending on the toxicant. Furthermore, these effects cannot be predicted by simply summing the effects caused by UV and by the toxicant when tested independently. This implies that it would be impracticable to apply a safety factor that is based on the a priori known sensitivity of *D. magna* to UV (e.g. Huebner et al. 2006) to derive safe concentrations that reflect the effects of UV. The derivation of safe concentrations that take into account the effects of UV exposure requires laboratory tests that can be implemented easily and standardised on the basis of existing standard methods, similar to the standard 21-days chronic reproduction test (OECD 1997) that was modified for use in the present study.

When the daphnids were exposed to UV, significant alterations in the population growth rate were found at concentrations of pirimicarb and fenoxycarb that were 3 and 20 times lower than the acute LC50s, respectively. Under field conditions such alteration of the population growth rate would result in a reduction in abundance, which can be further exacerbated by biotic stress (Beketov and Liess 2006; Rohr et al. 2008). However, because of the very limited number of concentrations tested, the present study cannot be used to quantify the extent to which UV radiation can exacerbate the effects of pesticides; for this, further investigations with a wide range of concentrations are necessary.

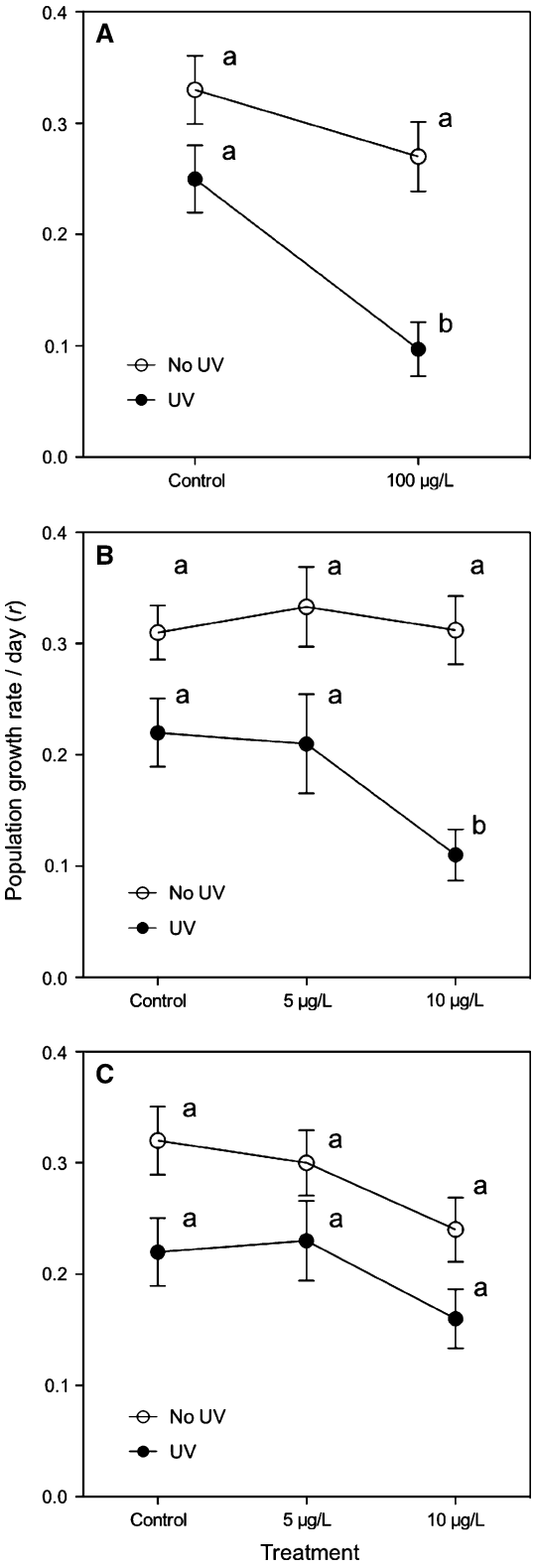

Fig. 2 Population growth rate (r) of *Daphnia magna* in the 21-d toxicity tests with exposure for 24 h to fenoxycarb (**a**), pirimicarb (**b**), or tebufenpyrad (**c**), with and without UV exposure. Values that are not significantly different from each other are marked with the same letter ($p < 0.05$)

With regard to the mechanisms that underlie the observed synergistic effects, two different processes can be proposed: (1) UV radiation could trigger photoactivation of the compounds inside the bodies of the tested daphnids after exposure to the chemicals (but not in the tested media), or (2) UV radiation could increase the individual sensitivity of the tested daphnids to the pesticides. The first explanation, although it cannot be rejected completely, seems less probable than the second one because it is not supported by existing empirical data. Also, UV has been shown repeatedly to cause photodegradation of different pesticides, which include the pesticides studied here (e.g. Pirisi et al. 1996); therefore, photodegradation, associated with a decrease of compound toxicity, might also be expected to have occurred in the present experiments. The second explanation is supported well by existing knowledge. It is known that in general nonchemical stressors that reduce individual fitness and cause mortality also increase individual sensitivity to toxicants (Heugens et al. 2001). Specifically, such combined effects have been shown previously for the combined effect of UV radiation, food deficiency and copper contamination, increasing the sensitivity of the crustacean *Paramoera walkeri* in microcosms by a factor more than 30 when compared to copper exposure alone (Liess et al. 2001). Additionally, the combination of these factors increased the sensitivity of this species in the wild (Duquesne and Liess 2003). Also, it has been shown that UV can increase the toxicity of sodium pentachlorophenate salt to the rotifer *Brachionus calyciflorus* as much as five-fold in experiments where photoactivation did not occur (Preston et al. 1999). Furthermore, the negative effects of UV on the reproduction and survival of *D. magna* have been described well in the literature (Huebner et al. 2006, for *D. pulicaria* see Williamson et al. 2001a), and were confirmed by the present study. Taken together, these findings suggest that the observed synergistic effects likely arise from the combined effect of the two stressors on daphnid physiology, rather than on the photoactivation of the pesticides inside the organisms. However, further studies are necessary for validation of these possible mechanisms.

In conclusion, the results of the present study show that UV radiation and pesticides in combination can have synergistic effects that cannot be predicted by simple summation of the independent effects of these stressors. However, the occurrence of such synergistic effects depends on the specific compound and its concentration. Given the current state of knowledge, this precludes cross-compound generalisation and the quantification of dose–response dependences. Such synergistic effects should and can be investigated experimentally in order to define realistic safe concentrations of pesticides and other toxicants in the environment. The present study also indicates

Table 2 Cumulative per capita reproduction of *Daphnia magna* in the 21-d toxicity tests with exposure for 24 h to fenoxycarb, pirimicarb, or tebufenpyrad and with or without UV exposure (number of neonates per individual, means and standard errors in parentheses)

Pesticide	Treatment, per capita reproduction (number of neonates)					
	No UV			UV		
	Control		100 μg/L	Control		100 μg/L
Fenoxycarb	107.9 (3.27)a		95.14 (5.87)ab	89.21 (3.95)b		70.62 (3.27)c
	Control	5 μg/L	10 μg/L	Control	5 μg/L	10 μg/L
Pirimicarb	105.2 (3.26)a	111.7 (3.91)a	112.2 (4.13)a	88.18 (3.48)b	90.67 (6.22)b	74.86 (3.19)c
Tebufenpyrad	111.1 (3.31)a	105.6 (2.91)a	95.54 (5.3)ab	87.92 (3.53)b	93.18 (4.9)ab	85.36 (4.93)b

Values that are not significantly different from each other are marked with the same letter (comparison within tests for each pesticide, $p < 0.05$)

that the synergistic effects of UV and pesticides may explain, at least partially, the differences in the effective concentration levels between field studies (e.g. Liess and von der Ohe 2005) and standard laboratory toxicity tests (for the cross-system analysis see Beketov et al. 2008). Furthermore, it demonstrates explicitly that knowledge about the combined effects of toxicants and UV radiation is indispensable for realistically predicting the consequences of increased UV irradiance and pollution, and in general for ecological risk assessment under conditions of climate change.

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