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Photo-induced toxicity and oxidative stress responses in *Tigriopus japonicus* exposed to nitro-polycyclic aromatic hydrocarbons and artificial light

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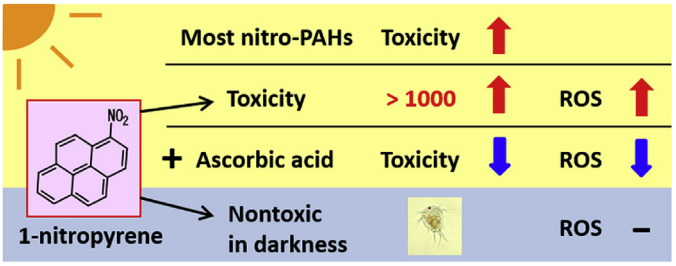
HIGHLIGHTS

- *T. japonicus* was exposed to 10 nitro-PAHs and low-intensity light irradiation.
- The toxicity of 9 nitro-PAHs was induced by the light irradiation.
- The toxicity of 1-nitropyrene was induced >1000 times higher than that in darkness.
- The toxicity of 1-nitropyrene increased with generation of ROS.
- Ascorbic acid suppressed generation of ROS and the toxicity of 1-nitropyrene.

GRAPHICAL ABSTRACT



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ABSTRACT

Photo-induced toxicity is an important phenomenon in ecotoxicology because sunlight reaches many organisms in their natural habitats. To elucidate whether sunlight enhances the toxicity of nitro-polycyclic aromatic hydrocarbons (nitro-PAHs), the acute toxicities of 10 nitro-PAHs and the related compound 1-nitropyrene (1-NP) to *Tigriopus japonicus* were assessed in darkness or under light conditions. In addition, the relationships among the toxicity of 1-NP to *T. japonicus*, lighting condition, and the concentration of reactive oxygen species (ROS) formed were investigated in the presence or absence of the ROS scavenger ascorbic acid in the test solutions. Light irradiation increased the toxicity of all tested nitro-PAHs except 1,5-dinitronaphthalene. Among the compounds tested, 1-NP was the most phototoxic: it was more than 1000 times more toxic under the light conditions than in darkness. In contrast, at the same light levels, pyrene was not phototoxic. Light irradiation induced the generation of ROS in the 1-NP exposure groups, and the immobilization rate of *T. japonicus* increased with the amount of ROS produced. The addition of ascorbic acid to the test solutions suppressed both the generation of ROS and the light-

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Photo-induced toxicity
Photodegradation products

induced immobilization of *T. japonicus*. To accurately assess the ecotoxicologic risk of nitro-PAHs, their overall photo-induced toxicity must be considered.

1. Introduction

Photo-induced toxicity occurs when the toxic effect of a chemical is induced or enhanced by visible or UV radiation (Ribeiro and Ferreira, 2005). Known photo-induced toxic chemicals include polycyclic aromatic hydrocarbons (PAHs) and PAH mixtures (Newsted and Giesy, 1987; Pelletier et al., 1997; Swartz et al., 1997; Huang et al., 1997), metals (Babu et al., 2003), pesticides (Ankley et al., 1998), nanoparticulate metal oxides (Dasari et al., 2013), and nitro-polycyclic aromatic hydrocarbons (nitro-PAHs; White et al., 1985; Kagan et al., 1990).

Various mechanisms of the photo-induced toxicity of PAHs have been reported. Photo-excitation of PAHs generates reactive species, primarily reactive oxygen species (ROS), PAH-derived free radicals, and lipid peroxides, all of which damage various cellular tissues and can lead to acute toxicity and genotoxicity (Fu et al., 2012; Lampi et al., 2005; Arfstena et al., 1996). The photo-induced toxicity of benzo[*a*]pyrene is inhibited by sodium azide (NaN_3), a scavenger of singlet oxygen (Ibuki and Goto, 2002). Photo-induced lipid peroxidation by PAHs is inhibited by scavengers of free radicals or singlet oxygen, and is enhanced by deuterium oxide, which prolongs the half-life of singlet oxygen (Xia et al., 2006). Ascorbic acid (AA) also works as a scavenger of ROS: it can quench free radicals as well as singlet oxygen (Machlin and Bendich, 1987).

Nitro-PAHs are formed directly or indirectly due to the incomplete combustion of PAHs (Nielsen, 1984) or during the interaction of PAHs with atmospheric nitrogen oxide (Atkinson and Arey, 1994). These compounds decompose in the atmosphere or are eliminated through wet or dry deposition; they are widely distributed in the environment, including aquatic systems (Bamford and Baker, 2003). Both PAHs and nitro-PAHs have been detected in marine sediments (Ozaki et al., 2010; Uno et al., 2011; Huang et al., 2014a), river water (Ohe and Nukaya, 1996; Murahashi et al., 2001), seawater (Murahashi et al., 2001), and aquatic organisms (Uno et al., 2011; Huang et al., 2014b).

Because nitro-PAHs may become phototoxic to aquatic organisms, the assessment of their associated hazards needs to reflect the contribution of environmental light levels to their photo-induced toxicity on marine organisms. The acute toxicity of various nitro-PAHs has been reported for some aquatic organisms. For example, the effects of 1-nitronaphthalene have been studied in the fathead minnow, *Pimephales promelas* (Curtis and Ward, 1981), and the ciliate *Tetrahymena pyriformis* (Schultz and Moulton, 1985). Another study assessed the acute toxicity of 10 different nitro-PAHs under white fluorescent lights or in darkness at three trophic levels on the mummichog *Fundulus heteroclitus*, the marbled sole *Pleuronectes yokohamae*, the copepod *Tigriopus japonicus*, and the diatom *Skeletonema costatum* (Onduka et al., 2012). Although a few reports address the photo-induction of nitro-PAH toxicity to human erythrocytes, *Escherichia coli* strains, and *Salmonella typhimurium* strains (White et al., 1985; Kagan et al., 1990), no studies regarding the phototoxicity of nitro-PAHs to marine organisms have been published, and the information currently available is insufficient to assess the hazards of increased toxicity of nitro-PAHs due to their exposure to environmental light conditions.

Our aim here was to elucidate how the toxicity of nitro-PAHs to marine organisms changed due to irradiation with levels of light similar to those in the environment. The copepod *T. japonicus* has been used as a model system in ecotoxicology studies; its natural habitat is tidal pools, where sunlight reaches the organism (Raisuddin et al., 2007). We therefore selected this copepod as a test organism in which to elucidate changes in toxicity due to light irradiation. We tested the acute toxicity of 10 nitro-PAHs on *T. japonicus* in the dark or under low-intensity light irradiation. In addition, we measured the degradation products of 1-nitropyrene (1-NP) that were induced by irradiation with high-intensity light and used these products and their related compounds 1-hydroxypyrene, 1-aminopyrene, and pyrene in acute toxicity tests on *T. japonicus*. Furthermore, we investigated the relationships among the toxicity of 1-NP to *T. japonicus*, lighting condition, and the concentration of ROS formed due to irradiation of 1-NP in the presence or absence of the ROS scavenger AA.

2. Materials and methods

2.1. Chemicals and organisms

We obtained 1-nitronaphthalene (purity, 99%), 2-nitrofluorene (purity, 98%), 3-nitrofluoranthene (purity, 90%), 3-nitrophenanthrene (purity, 97%), 6-nitrochrysene (purity, 95%), 1,3-dinitropyrene (purity, 99%), and 1,6-dinitropyrene (purity, 98%) from Sigma–Aldrich (St. Louis, MO, USA); and 1-NP (purity, >98%), 1,5-dinitronaphthalene (purity, 97%), and 2,7-dinitrofluorene (purity, >95%) from Tokyo Chemical Industry (Tokyo, Japan). Pyrene (purity, 98.0%), 1-hydroxypyrene (purity, 97.0%), 1-aminopyrene (purity, 97%), and AA (purity, 99.6%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). For toxicity tests, stock solutions (20 mM) of these test chemicals were prepared in acetone (analytical grade, Wako Pure Chemical Industries). Test solutions were obtained by diluting the stock solutions with seawater filtered sequentially through sand, activated carbon, and finally a glass-fiber cartridge filter (GF/C filter, Whatman, Maidstone, UK; 31 ± 1 ppt salinity, mean ± standard error of the mean [SEM]; hereafter referred to as “filtered seawater”).

Adults of the copepod *T. japonicus* initially were obtained from the Marine Ecology Research Institute (Tokyo, Japan), and then raised in our laboratory. The animals were cultured in 800 mL of filtered seawater in a 1-L glass vessel with slight aeration. This stock culture was maintained under the conditions of a 14:10-h light:-dark photoperiod and 20 ± 1 °C in a glass growth chamber (MLR-350, Sanyo, Osaka, Japan) and fed a diet of the prasinophyte *Tetraselmis tetrathele* cultured in f/2 medium (Guillard and Ryther, 1962) prepared from filtered seawater. Algal cells were added to the stock culture (>10^4 algal cells/mL) once daily. The copepod culture was filtered with a nylon screen (NB80 [mesh size, 190 μm]; NBC Meshtec, Tokyo, Japan) to remove the adults, and the filtrate was further filtered with a fine mesh nylon screen (N-NO305T [mesh size, 48 μm]; NBC Meshtec). Copepod nauplii (<24 h old) obtained on the mesh were used for the immobilization tests described later.

2.2. Photoirradiation

Illumination experiments were performed in the glass growth