PRELIMINARY ASSESSMENT OF TNT-EXPOSURE IN FISH IN THE TREMITI ISLAND MARINE PROTECTED AREA

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Abstract

This study was performed to preliminarily assess the ecological risk due to the presence of 2,4,6-trinitrotoluene and its degradation products in the marine environment. A multimarkers approach (EROD, AChE) was applied in specimens of *Conger conger* (Linnaeus, 1758) collected in the waters of Pianosa Island (TNT site) and in a reference site, S. Domino Island, in the same archipelago, the Tremiti Islands Marine Protected Area (Adriatic Sea, Italy). Levels of TNT in fish tissues were also measured.

Keywords: biomarkers, TNT, Tremiti Islands, biomonitoring

Introduction

Disposal at sea of ordnance containing the explosive 2,4,6-trinitrotoluene (TNT) represents a serious hazard to marine ecosystems (1). The objectives of the present study were to obtain preliminary data regarding the health of demersal fish species resident in a TNT-impacted site and to test the possibility of using the sentinel fish species *Conger conger* (Linnaeus, 1758) as a suitable bioindicator to monitor the biological effect of TNT-exposure in aquatic organisms.

Within the current research, Pianosa Island (Tremiti Islands Marine Protected Area, Adriatic Sea, Italy) was chosen as the study area because the seabed around the island has served as a dumping ground for bombs since the Second World War.

Both a chemical approach, to detect traces of these products in fish tissues, and a biomonitoring approach, aimed at detecting enzymatic alterations by means of specific biomarkers, were applied.

Two well-established enzymatic responses currently used in pollution monitoring and assessment were chosen: the 7-ethoxyresorufin-O-deethylase (EROD) and Acetylcholinesterase (AChE).

Materials and methods

Specimens of *C. conger* were collected in June 2001 and 2002 both in Pianosa Island and in a reference site in the same archipelago near S. Domino Island (Tremiti Islands Marine Protected Area, Adriatic Sea, Italy). The Fish tissues were examined for presence of TNT, 4ADNT and 2ADNT traces by means of a chromatographic assay (2), as well as liver microsomal EROD activity (3), and Acetylcholinesterase (AChE) activity in brain tissues (4). Total protein contents were measured (5). Statistical analyses were carried out using STATISTICA® *Stat Soft* 5.0 software. Significant differences between enzymatic values were assessed using the non parametric Mann-Whitney test and results were considered as statistically significant at $p<0,05$.

Results

No detectable levels of TNT or its degradation products were observed in *C. conger* tissues by means of the chromatographic assay. AChE activities assayed in the brain were significantly lower in specimens collected from the TNT-impacted site in both 2001 and 2002 compared to those measured in specimens collected in the reference site. Significantly higher EROD activities were observed in samples from the impacted area compared to those collected from the reference site (Fig. 1).

The results of this preliminary study reveal a situation of instability of the fish enzymatic systems. The lower AChE activities in specimens from the TNT-impacted site suggest the presence of neurotoxic compounds, like TNT (6), able to affect nerve impulse transmission.

Moreover, the higher EROD activities observed in specimens from the impacted site suggest potential biotransformation pathways for TNT involving P-450 enzymes, as already suggested in previous studies (7-9).

The metabolic alterations registered within the specimens collected from Pianosa Island should be considered as a sign of exposure to toxic compounds. However, the lack of data concerning the presence of TNT or its degradation products within the tissues of the same specimens, stresses the need of further information to verify the responsibility of these products in altering enzymatic activities.

The current research presents preliminary data concerning biomarkers of exposure and effect in *C. conger*, potentially very useful for future ecotoxicological studies in the marine environment.

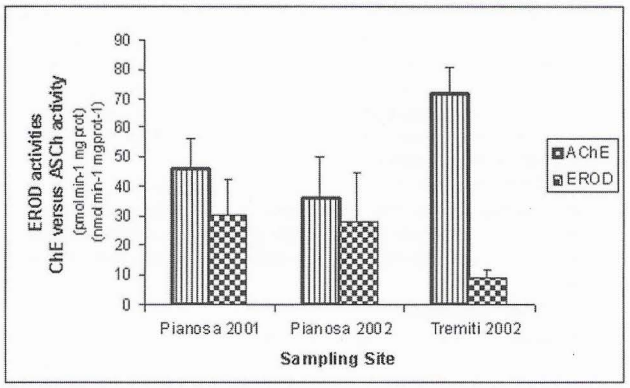

Fig. 1. EROD activities (pmol min-1 mg prot-1) in liver and ChE activity *versus* ASCh (nmol min-1 mg prot-1) in brain of *C. conger* collected in the Tremiti Islands Marine Protected Area (Adriatic Sea, Italy). Values are expressed as mean ± standard deviation (6<n<10).

Conclusions

The absence of detectable traces of TNT and its degradation products in fish tissues confirms their low biodisponibility and bioconcentration potential. The results of the multimarkers approach however, taking into account the absence of local xenobiotics sources in the uninhabited and protected island, reveal the existence of a stressing condition that could be linked to the presence of these molecules in the impacted site.

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