Aquatic Toxicology 78S (2006) S105–S108

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Induction of micronuclei and other nuclear abnormalities in mussels exposed to bisphenol A, diallyl phthalate and tetrabromodiphenyl ether-47

Janina Baršienė [a,*], Janina Švyokienė [a], Anne Bjornstad [b]

[a] *Institute of Ecology of Vilnius University, Akademijos 2, 08412 Vilnius, Lithuania*
[b] *IRIS, International Research Institute of Stavanger AS, Mekjarvik 12, N-4070 Randaberg, Norway*

Abstract

Analysis of micronuclei, nuclear buds, bi-polynucleated and fragmented-apoptotic cells was performed in gills of blue mussels exposed for 3 weeks to sublethal concentrations of bisphenol A, diallyl phthalate (for the both nominal concentration 50 ppb) and to tetrabromodiphenyl ether-47 (nominal concentration 5 ppb). Fourteen specimens from each treatment and control group were used for the analysis. Our results demonstrated a significant increase in micronuclei frequency after the treatment with bisphenol A ($P = 0.0243$), diallyl phthalate ($P = 0.0005$) and tetrabromodiphenyl ether-47 ($P < 0.0001$; Mann–Whitney U-test). Induction of bi-nucleated ($P = 0.0028$), fragmented-apoptotic ($P = 0.0004$) cells and nuclear buds ($P = 0.0101$) was found in mussels exposed to tetrabromodiphenyl ether-47 while treatment with diallyl phthalate increased the level of fragmented-apoptotic cells ($P = 0.0283$). Bisphenol A was the only agent that resulted only in induction of micronuclei but not any other kind of nuclear injuries.

Keywords: Micronuclei; Mussells; Bisphenol A; Diallyl phthalate; Tetrabromodiphenyl ether-47

1. Introduction

There are many compounds that induce genotoxic effects with or without directly damaging DNA. Micronuclei can be produced from chromosomal fragments or whole chromosomes that lag at cell division due to lack of centromere, damage in centromere or defect in cytokinesis. These small secondary structures of chromatin are surrounded by membranes, located in cytoplasm and have no detectable link to the cell nucleus (Heddle, 1973; Heddle et al., 1991). The micronuclei (MN) test has been developed primarily in mammalian cells (Boller and Schmid, 1970; Heddle, 1973) and later has been adopted for the assessment of cytogenetic damage in different organisms including marine mussels from the *Mytilus* genus (Majone et al., 1987, 1988, 1990; Venier et al., 1997; Bolognesi et al., 1999, 2004; Baršienė et al., 2004).

The occurrence of other nuclear abnormalities—nuclear buds, binucleated and fragmented-apoptotic cells have also been considered as reliable approach in assessment of genotoxic and cytotoxic effects of contaminants in molluscs (Venier et al., 1997; Dailianis et al., 2003). Nuclear buds develop from the eliminated extra-chromosomally amplified DNA (Shimizu et al., 1998, 2000), as well as potentially from defective DNA-repair complexes (Haaf et al., 1999). Apoptosis is genetically determined death of cells, that can be provoked by variety of factors, including those with genotoxic potential. Some publications present the evidence of apoptosis induction in molluscs caused by chemical compounds (Steinert, 1996; Steinert et al., 1998; Mičic et al., 2001; Bihari et al., 2003).

In this paper we report the data on the frequency of micronuclei, nuclear buds, bi-nucleated and fragmented-apoptotic cells in gill cells of the blue mussels exposed to endocrine disruptors bisphenol A and diallyl phthalate, as well as to flame retardant tetrabromodiphenyl ether (BDE-47) under experimental conditions. Bisphenol A, a monomer of polycarbon plastics and epoxy resins, is found in food-packaging, can-coating chemicals and in dental sealants. This compound may be released into food, reach circulating blood and can cause genotoxic and cytotoxic effects. Disruption of the mitotic spindle, induction of metaphase arrest and micronuclei containing whole chromosomes/chromatids has been determined in human, mouse and Chinese hamster cells (Pfeiffer et al., 1997; Suarez et al., 2000; Lehmann and Metzler, 2004; Masuda et al., 2005). Polybrominated diphenyl ethers (PBDEs), used as flame retardant, are widely distributed in wildlife samples from Europe, Australia, North America and the Arctic (Law et al., 2003) and

* Corresponding author. Tel.: +370 6 8260979; fax: +370 5 2729257.
E-mail address: janbar@ekoi.lt (J. Baršienė).

0166-445X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2006.02.023

their high persistence raises an environmental concern that is likely to be serious problem in the future. PBDEs have been detected in mollusc tissues (Gustafsson et al., 1999; Booij et al., 2002; Christensen et al., 2002). The blue mussels inhabiting the Baltic Sea rapidly uptake PBDEs resulting in high levels accumulated (Gustafsson et al., 1999; Booij et al., 2002; Baršienė et al., 2006). Only fragmentary data exist on PBDEs mutagenic and clastogenic action (Evandri et al., 2003). There is no data on genotoxicity of diallyl phthalate.

2. Materials and methods

2.1. Experimental treatment

Mussels were collected from clean reference site Forlandsfjorden and acclimated in laboratory conditions (RF-Akvamiljo experimental laboratory, Stavanger, Norway) maintaining them in filtrated sea water for 7 days prior to experimentation. Sea water was pumped from 80 m depth. In flow through system, mussels in 600 l glass fiber tanks were exposed to sublethal concentrations of bisphenol A, diallyl phthalate (for the both nominal concentration 50 ppb) and to tetrabromodiphenyl ether-47 (nominal concentration 5 ppb). Mussels received natural daylight and during night, the tanks were covered to protect organisms from artificial light. Crude oil was pumped from a reservoir column (*Pharmacia* 56-1190-30) to the mixing unit and oil emulsion was distributed in appropriate concentrations to the experimental tanks using *Watson Marlow 505 U* peristaltic pump. After 3 weeks of exposure, gill samples were collected from 56 mollusc specimens. Fourteen specimens from each treatment and control group were used for the analysis. Experimental details are reported in Sundt et al. (2006).

2.2. Preparation and analysis of slides

Two gill arches of mussel were placed in a big drop of 3:1 ethanol acetic acid (or methanol acetic acid) solution separately on two clean microscopic slides and gently nipped with tweezers for 2–3 min (until cells spread within a drop). Then the cell suspension was softly smeared on both slides. Dried slides were fixed in methanol for 10 min and stained with 4% Giemsa solution in phosphate buffer pH 6.8. The stained slides were analyzed under the light microscope Olympus BX51 at a final magnification of 1000×. For each studied specimen of mussels, 2000 cells with intact cytoplasm were scored (Baršienė et al., 2004). The frequency of micronuclei and other nuclear abnormalities was expressed as the number of MN/nuclear abnormalities per 1000 cells scored.

2.3. Analysis of micronuclei and other nuclear abnormalities

The blind scoring of micronuclei and other nuclear abnormalities was performed on coded slides without knowledge of the origin of samples. Only cells with intact cellular and nuclear membrane were scored. Round or ovoid-shaped non-refractory particles with colour and structure similar to chromatin, with a diameter 1/3–1/20 of the main nucleus and clearly detached from it were interpreted as micronuclei (Fig. 1). In general, colour intensity of MN should be the same or less than of the main nuclei. Nuclear buds, bi-nucleated and fragmented-apoptotic cells were identified following criteria described by Fenech et al. (2003).

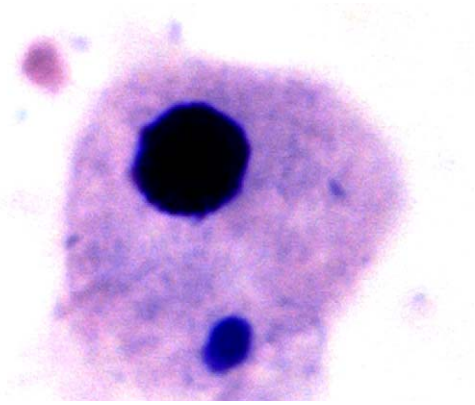

Fig. 1. Micronucleus in gill cell of blue mussel.

The statistical analysis was carried out using PRISM statistical package. Mean and standard error was calculated for each experimental group. Non-parametric Mann–Whitney *U*-test was used to compare MN and other nuclear abnormalities frequencies between control and treatment groups.

3. Results

3.1. Induction of MN

The mean frequency of MN in the gills of the control mussel group was 0.97 MN/1000 cells while the PBDE-47 exposure resulted in MN value of 3.96 MN/1000 cells (Fig. 2). The exposure to bisphenol A and diallyl phthalate induced the MN

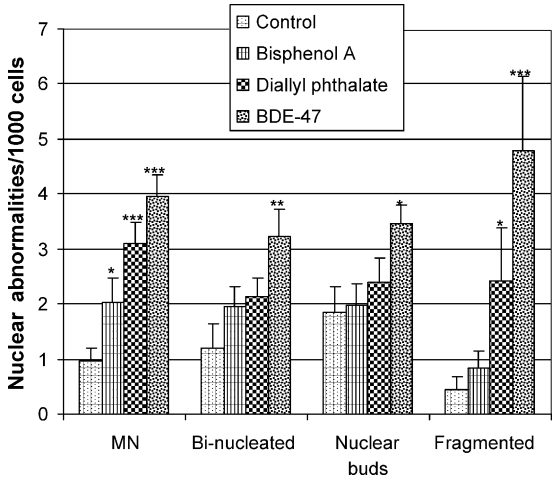

Fig. 2. Frequency of micronuclei (MN), bi-nucleated cells, nuclear buds and fragmented-apoptotic cells in gills of mussels exposed to bisphenol A, diallyl phthalate and tetrabromodiphenyl ether-47. Asterisks show statistically significant differences compared to control group ($^{*}P$ at 0.05, $^{**}P$ at 0.001, $^{***}P$ at 0.0001 levels).