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Induction of micronuclei and other nuclear abnormalities in blue mussels *Mytilus edulis* after 1-, 2-, 4- and 8-day treatment with crude oil from the North Sea

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The genotoxicity and cytotoxicity of crude oil processed from the Statfjord B platform in the North Sea were studied in gills of blue mussels (*Mytilus edulis*), exposed to 0.5 ppm concentration of the dispersed oil for 1, 2, 4 and 8 days. Induction of micronuclei (MN) and nuclear buds (NB) was assessed as the crude oil genotoxicity endpoints; induction of fragmented-apoptotic (FA) and bi-nucleated (BN) cells indicated the cytotoxicity potential of the oil. Time-related MN elevation incidences were detected in all experimental groups compared to the control group of mussels. The elevation of micronuclei increased progressively with increasing the duration of exposure – from 1.9-fold after 1 day to 2.4-fold after 8 days of exposure. There was no significant occurrence of nuclear buds in the experimental groups. A significant induction of fragmented-apoptotic cells ($P = 0.0115$) was detected in *M. edulis* gills after a 4-day exposure and of bi-nucleated cells after an 8-day treatment ($P = 0.0232$).

Key words: micronuclei, nuclear abnormalities, crude oil, blue mussel

INTRODUCTION

Environmental contaminants released from oil industry are well known as the primary source of persistent toxicity in aquatic ecosystems. Certain substances, such as crude oil, polycyclic aromatic hydrocarbons, heavy metals and alkylphenols, are usually present in petroleum industry wastewater and attract particular attention because of their potential mutagenic and carcinogenic properties. The hazardous substances may occur below the detection limit; however, they act as genotoxins, inducing genomic instability at very low concentrations. Furthermore, contaminants, usually discharged in complex mixtures, can provoke interactions among unknown substances and lead to deviations in genotoxicity responses to pollution (Jha, 2008). Therefore, the need to develop and standardize tests for the monitoring of their biological effects is urgent in polluted marine ecosystems. This poses a major challenge for petroleum industry managers to protect indigenous populations and provide methodological instruments to monitor the effects of hazardous substances. Marine organisms could be exposed to genotoxic compounds via several routes. Genotoxins can bind to DNA molecules and trigger off a damaging chain of biological changes, such as an impaired enzyme function or general metabolism, cytotoxicity, immunotoxicity, reproduction disturbances, growth inhibition, or carcinogenesis (Ohe et al., 2004). Oil spills can result in a wide distribution of petroleum hydrocarbons in the marine environment, seriously impacting DNA of filter-feeding bivalve populations (Hamoutene et al., 2002). Bivalves have a limited ability to metabolize petroleum hydrocarbons and accumulate comparatively high levels of these compounds in their tissues (Dyrynda et al., 1997). Hazardous effects of various polyaromatic hydrocarbons (PAHs) arise typically as a result of oxidative biotransformation producing highly DNA-reactive metabolites. These metabolites are recognized as carcinogenic and mutagenic compounds (Woodhead et al., 1999).

The micronucleus (MN) test, one of those most frequently used in environmental genotoxicity studies, has served as an index of cytogenetic damage for over 30 years (Fenech et al., 2003). This is a sensitive and fast test to detect genomic alterations due to clastogenic effects and impairments of mitotic spindle. The MN test was originally developed for the analysis of chemical genotoxicity in mammals (Heddle et al., 1991), and later it has been successfully adapted to species from other groups, including aquatic organisms. Micronuclei

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can be produced from chromosomal fragments or the whole chromosome that lags during cell division due to the lack of a centromere, damage in a centromere or a defect in cytokinesis (Heddle et al., 1991).

Increased MN levels have been described in zones affected by oil spills (Parry et al., 1997; Harvey et al., 1999; Baršienė et al., 2004, 2006a, 2006b). A significant elevation of genotoxicity was observed in mussels 30 days post-oil-spill, and the persistence of the cytogenetic damage lasted up to 100 days (Parry et al., 1997) or even 7 months (Baršienė et al., 2006a). A statistically significant increase of micronuclei levels was found in oysters and fish caged in Haven oil spill zones 10 years after an oil spill (Bolognesi et al., 2006a). Increased genotoxicity was described in mussels from oil terminal and marine port zones in the Baltic Sea (Baršienė, Baršytė Lovejoy, 2000; Baršienė, 2002), in a Mediterranean commercial port zone (Magni et al., 2006), in zones of the Venice lagoon, polluted by aromatic hydrocarbons (Venier, Zampieron, 2005), coastal Mediterranean ecosystems in Croatia (Klobučar et al., 2008), after dredging contaminated by PAHs and Pb sediments (Bocchetti et al., 2008).

A positive relationship between MN frequencies and PAH concentrations (mainly alkylated homologues) was described in inter-tidal mussels *Perna perna* from a Brazilian coast chronically contaminated with oil, particularly after the major MF–380 oil spill in January 2000. In the PAH profile, the predominance of petrogenic compounds, especially phenanthrenes, was identified. Significant elevations of MN incidence were observed for ΣPAH above 1000 μg/kg, and the levels close to or above 300 μg/kg are able to initiate an increase in MN frequency (Francioni et al., 2007).

In addition to the MN test, nuclear buds, fragmented-apoptotic, bi-nucleated cells and some other nuclear abnormalities were successfully used for assessing the pollutant effects in mussels (Venier et al., 1997; Dolcetti, Venier, 2002; Izquierdo et al., 2003; Carvalho Pinto Silva et al., 2005; Venier, Zampieron, 2005; Baršienė, Rybakovas, 2006; Baršienė et al., 2006a, 2006b, 2006c, 2006d, 2008; Baršienė, Andreikėnaitė, 2007; Koukouzika, Dimitriadis, 2008). Significantly increased levels of micronuclei, nuclear buds and fragmented apoptotic cells were found in bivalves inhabiting the Baltic Sea close to the Būtingė oil terminal (Baršienė et al., 2006a) and to the Russian oil platform D-6 (Baršienė et al., 2008).

Formation of nuclear buds may reflect the unequal capacity of organisms to expel damaged, amplified, failed replication or improperly condensed DNA, chromosome fragments without telomeres and centromeres from the nucleus (Lindberg et al., 2007). Elimination of cytogenetic damage by apoptosis and necrosis is a key process which occurs at different rates in various organisms (Micič et al., 2002). Nevertheless, there is an information gap in our knowledge on formation of nuclear buds and bi-nucleated cells in marine organisms, which could be useful markers for pollution effects assessment and for validating the battery of the genotoxicity and cytotoxicity test in aquatic media.

Despite progress in the development of biomarker approach, there is a lack of laboratory-controlled studies with environmentally realistic doses of genotoxic and cytotoxic compounds used to reflect their damage formation in indigenous species inhabiting chronically contaminated oil platform areas. Recent findings of high levels of micronuclei and other nuclear abnormalities found in fish and mussels after a 3-week exposure to crude oil processed from the Statfjord B platform (Baršienė et al., 2006e; Bolognesi et al., 2006b; Baršienė, Andreikėnaitė, 2007) has raised concerns as to the environmental genotoxicity and cytotoxicity of crude oil from the North. An experimental treatment with 0.5 ppm of the Statfjord B crude oil significantly increased the incidence of micronuclei ($P = 0.0288$) and fragmented-apoptotic (FA) cells ($P = 0.0115$). The co-exposure to 0.5 ppm of oil spiked with a mixture of alkylphenols ($\Sigma = 0.1$ ppm) and 0.1 ppm of PAHs induced the highest level of MN (up to 3.6‰; a 2.8-fold increase versus the control level), nuclear buds (up to 1.5‰; a 4-fold increase versus the control) and FA cells (up to 2.4‰; a 4-fold increase versus the control level) (Baršienė, Andreikėnaitė, 2007). Increased MN levels were observed after caging Atlantic cod (*Gadus morhua*) and blue mussels (*Mytilus edulis*) in the areas of oil platforms or in indigenous fish species inhabiting the oil platform areas in the North Sea (Hylland et al., 2008; our unpublished data).

The main objective of the present study was to evaluate genotoxicity and cytotoxicity in mussels *Mytilus edulis* after a 1-, 2-, 4- and 8-day treatment with 0.5 ppm crude oil processed from the Statfjord B platform in the North Sea. Induction of micronuclei (MN) and nuclear buds (NB) in gills was used as genotoxicity endpoints, while induction of binucleated (BN) and fragmented-apoptotic (FA) cells in gills of *M. edulis* was utilized as cytotoxicity endpoints.

MATERIALS AND METHODS

Experimental treatment

Blue mussels (*Mytilus edulis*) were colleted in Førlandsfjorden (Southern Norway). This location had been frequently used as a reference site in previous ecotoxiocological studies (e. g., Baršienė et al., 2004, Baršienė, Andreikėnaitė, 2007). Following 9 days of acclimation, the mussels were exposed to a continuous flow system at the IRIS Biomiljø, Norway (as described by Sundt et al., 2006) for 1, 2, 4 and 8 days, to a nominal concentration of 0.5 ppm of dispersed North Sea crude oil (i. e. Statfjord B oil). Control mussels received only filtered seawater (salinity 34‰, temperature 10 °C). Oil dispersions were made mechanically by passing oil and seawater through a high pressure mixing valve. The composition of PAHs in Statfjord B oil is listed in Table.

Ten specimens were used for the genotoxicity and cytotoxicity analysis from each of experimental and control groups. Via the flow through system, 10 litres/min of the sea water for all groups, i. e. more than one litre/kg biomass/min, was created in 600-litre glass fiber tanks. A *Watson Marlow 2 058*

Table. **The PAH composition recorded in Statfjord B crude oil by GC-MS and some characteristics of the PAH molecules**

Compound / quantity	Statfjord µg/g oil	Mass (M/Z)	Log K_{ow}
Naphthalene	1 147.0	128.2	3.3
C1-Naphthalenes	3 787.3	142.2	3.9
C2-Naphthalenes	5 288.7	156.2	4.4
C3-Naphthalenes	3 830.3	170.2	4.9
Acenaphthylene	10.0	152.2	4.1
Acenapthene	9.7	154.2	4.0
Fluorene	135.9	166.2	4.2
Anthracene	252.9	178.2	4.6
Phenanthrene	–	178.2	4.6
C1-Phenanthrenes	460.2	192.2	5.1
C2-Phenanthrenes	439.4	206.0	–
Dibenzothiophene	91.9	184.2	4.4
C1-Dibenzothiophene	196.5	198.2	–
C2-Dibenzothiophene	232.9	212.2	–
Fluoranthene	2.6	202.0	5.1
Pyrene	8.6	202.0	5.1
Chrysene	23.9	228.2	5.7
C1-Chrysene	37.9	242.2	–
C2-Chrysene	41.5	256.2	–
Benzo(a)anthracene	3.3	228.2	5.7
Benzo(b)fluoranthene	7.7	252.3	6.4
Benzo(k)fluoranthene	–	252.3	6.5
Benzo(b + k)fluoranthene	6.9	–	–
Benzo(a)pyrene	4.7	252.3	6.3
Indeno(1,2,3,cd)pyrene	–	276.3	6.9
Benzo(g,h,i)perylene	1.7	276.3	7.0
Dibenzo(a,h)anthracene	–	278.3	6.7
Sum PAH	16 020.9		
Sum PAH in 1 ppm dose (µg)	16.0		

Note. Log K_{ow} is the logarithm of the octano-to-water coefficient K_{ow}. LogK_{ow} values on alkylated PAH are hard to find. A thumb rule is to add from 0.3 to 0.5 log units per methyl group added; see also http://logkow.cisti.nrc.ca. The 1 ppm oil dosage would equal a quantity of 1 mg oil/kg seawater.

peristaltic pump with *Watson Marlow Maprene* 0.76 mm pump tubes was used for the crude oil calibration and intake into the tanks. The exposure components were introduced into the inflow of sea water in each tank. Suitable water currents, component's inflow speed and improved water circulation were adjusted by a nozzle.

Preparation of slides

A branch of mussel gills was placed in a big drop of 3 : 1 ethanol acetic acid solution on clean microscopic slide and gently nipped with tweezers for 2–3 min (until cells spread within a drop). Then the cell suspension was softly smeared on a surface of the slide. Dried slides were fixed in methanol for 10 min and stained with 5% Giemsa solution in phosphate buffer pH = 6.8. The stained slides were analyzed under the Olympus BX51 light microscope at the final magnification of 1000×.

Analysis of crude oil genotoxicity and cytotoxicity endpoints

The blind scoring of micronuclei and other nuclear abnormalities was performed on coded slides without knowledge of the origin of samples. Micronuclei (MN) were identified according to the following criteria: (1) round and ovoid-shaped non-refractory particles in the cytoplasm, (2) colour and structure similar to those of chromatin, (3) a diameter of 1/3–1/20 of the main nucleus, (4) particles completely separated from the main nucleus (Fig. 1). Nuclear buds, binucleated and fragmented-apoptotic cells were identified using the criteria described by M. Fenech with co-authors (2003). The morphological features of studied nuclear abnormalities are shown in Fig. 1. For each specimen of mussels, 2 000 cells with intact cytoplasm were scored. The frequency of micronuclei and other nuclear abnormalities was evaluated as the number of abnormalities per 1 000 cells scored (Baršienė et al., 2004).

Statistical analysis was carried out using the PRISM statistical package. The mean and the standard error were calculated for each experimental group. The non-parametric Mann–Whitney U-test was used to compare alteration frequencies between control and treatment groups. One-way ANOVA and post hoc Tukey's test were performed to determine whether significant differences were present among the treatment groups.

RESULTS

Crude oil genotoxicity (MN and NB)

The frequency of MN in gills of blue mussels varied from 1.4 MN/1000 cells in the pre-exposure group to 3.4 MN/1000 cells in those after an 8-day treatment with Statfjord B crude oil. Exposure time-dependent elevation of MN incidences was detected in mussels from 1-, 2-, 4- and 8-day treatment groups (Fig. 2). A statistically significant increase of MN values was found in mussels after a 1-day ($P = 0.0185$), 2-day ($P = 0.0039$), 4-day ($P = 0.0068$) and 8-day ($P < 0.0001$) exposure to crude oil. Lower levels of induction of nuclear buds compared to MN incidences were noted, and there were no statistically significant differences in nuclear bud frequency between pre-exposure and exposure groups. A comparatively low induction of nuclear buds (NB) was recorded in the 4-day treatment group (Fig. 2). One-way analysis of MN variance (ANOVA) with post hoc Tukey's test showed $P = 0.0033$, $F = 4.615$, whilst the analysis of NB variance showed $P = 0.2638$, $F = 1.357$.

Crude oil cytotoxicity (FA and BN)

The values of fragmented-apoptotic (FA) cells were lower than of nuclear buds. In the pre-exposure group, the level of FA was equal to 0.67 FA/1000 cells. After a 4-day exposure to crude oil, the value reached 1.43 FA/1000 cells and after a 2-day exposure 1.42 FA/1 000 cells (Fig. 2). Nevertheless, as was seen in the study results, significant differences

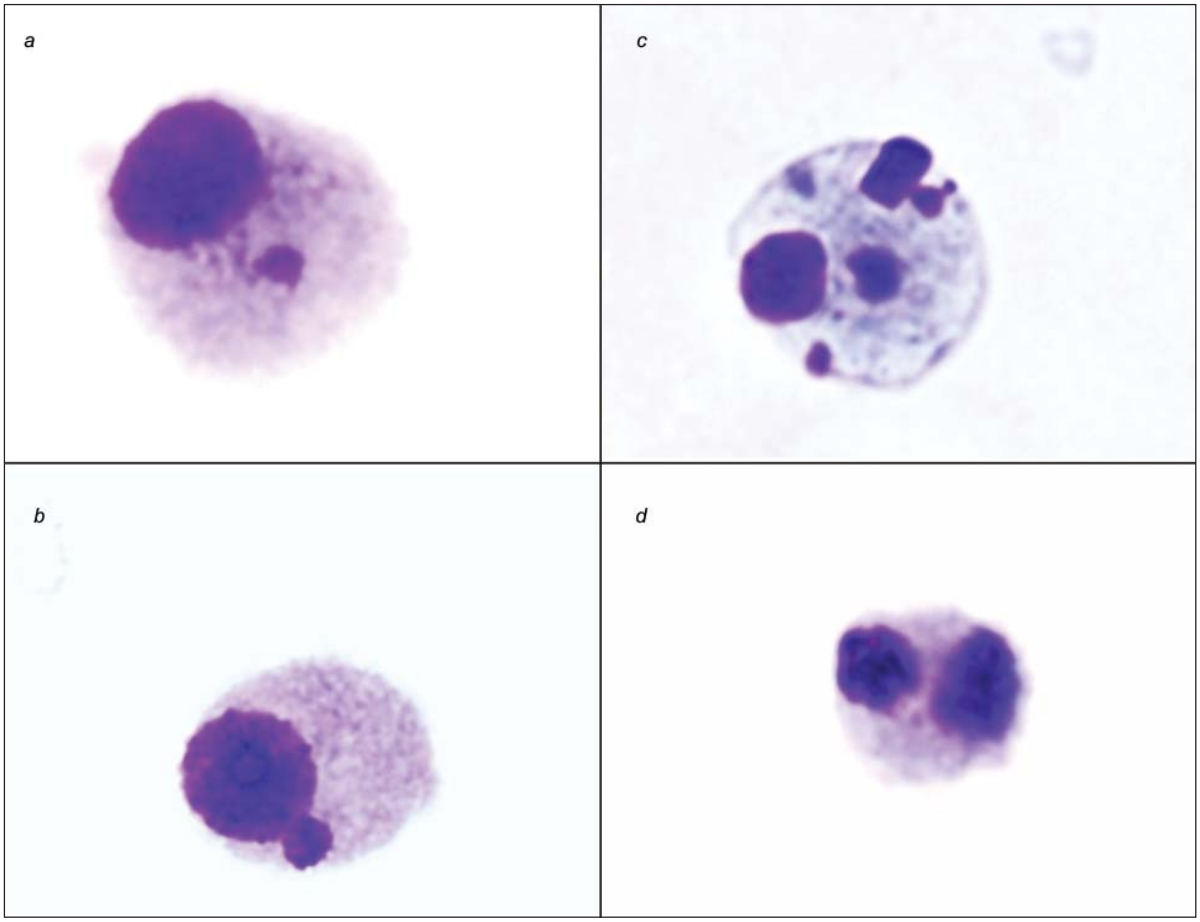

Fig. 1. *a* – micronucleus, *b* – cell with nuclear bud, *c* – fragmented-apoptotic cell, *d* – bi-nucleated cell in blue mussels gills

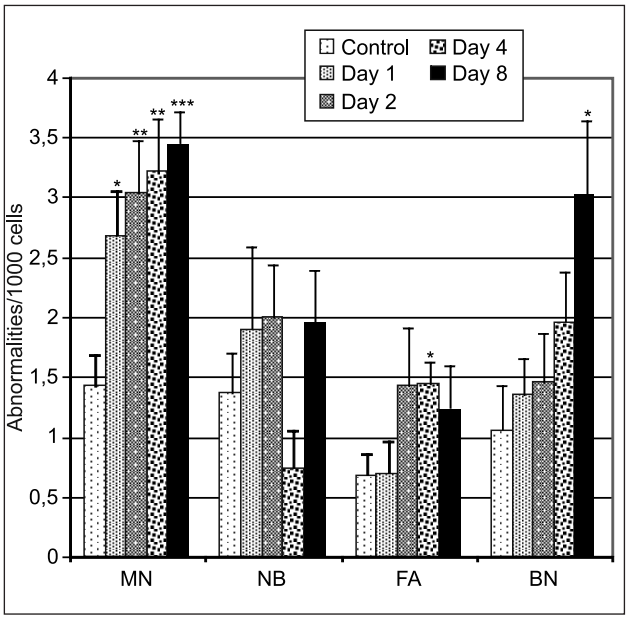

Fig. 2. Frequency of micronuclei (MN), nuclear buds (NB), fragmented-apoptotic (FA) and bi-nucleated (BN) cells in mussel gills exposed to 0.5 ppm of crude oil. Differences between pre-exposure and exposure groups: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$

(P = 0.0115) were found only between mussels from the 4-day oil treatment and the pre-exposure groups. The frequency of bi-nucleated (BN) cells ranged from 1.05 BN/1000 cells in the pre-exposure group to 3.02 BN/1000 cells in those after an 8-day oil treatment (Fig. 2). The BN levels showed the following trend: pre-exposure < 1-day < 2-day < 4-day oil exposure mussel groups. The non-parametric Mann–Whitney U-test showed that BN induction in gills of mussels after an 8-day exposure to Statfjord B oil significantly differed from the pre-exposure level (P = 0.0232). The one-way ANOVA analysis of BN variance, followed by the Tukey test, yielded P = 0.0266, F = 3.042; analysis of FA cells: P = 0.4636, F = 0.9148.

DISCUSSION

Offshore oil and gas installations are the major contributors to pollution in the North Sea. Water, crude oil, heavy metals, alkylphenols and other compounds are usually either co-produced or added during extraction processes in platforms (Røe, 1999). Our recent analysis of environmental genotoxicity within the water column monitoring station near the Statfjord B oil platform revealed a clear gradient-related MN increase in mussel haemocytes and liver erythrocytes of

Atlantic cod caged in 2004 for 6 week in the area of the platform. Significantly increased MN levels were detected in mussels and cod deployed 500 meters from the platform, compared to the reference site (Hylland et al., 2008). Nevertheless, there was no significant induction of nuclear buds, bi-nucleated and fragmented-apoptotic cells in mussel haemolymph and Atlantic cod liver erythrocytes (our unpublished data). In the Statfjord B platform area, the concentrations of PAHs and alkylphenols were low compared to the other coastal sites. Only the biomarkers used to monitor impacts such as EROD, DNA adducts, PAH metabolites, etc., lysosomal destabilization in mussel hepatopancreas and micronuclei induction in haemocytes indicated that caged mussels suffered from contaminants in the studied area of the North Sea and thus were validated as sensitive biomarkers to be used to monitor low levels of petroleum contaminants (Hylland et al., 2008).

In the present study, the genotoxicity and cytotoxicity of crude oil processed from the Statfjord B platform in the North Sea were studied in gills of mussels exposed to 0.5 ppm of oil for 1, 2, 4 and 8 days. Significantly higher MN frequencies were determined in all experimental groups compared to the pre-exposure group of mussels; an 1.9-fold elevation of micronuclei was found after a 1-day, 2.1-fold after a 2-day, 2.3-fold after a 4-day and 2.4-fold after an 8-day exposure. Therefore, a high potential of crude oil to quickly induce an irreversible DNA damage in mussels was revealed by the MN test. Nevertheless, in a short-term exposure, there was no significant increment of nuclear buds. Data of our previous study had shown that the capacity of mussels to expel a damaged amplified, failed replication or improperly condensed DNA, chromosome fragments without telomeres and centromeres from the nucleus appeared after a 3-week exposure of mussels to the Statfjord B crude oil (Baršienė, Andreikėnaitė, 2007).

Investigation of crude oil cytotoxicity revealed a significant incidence of fragmented-apoptotic cells in mussels after a 4-day exposure and bi-nucleated cells in mussel gills after an 8-day treatment. Thus, the results presented here confirm the genotoxicity and cytotoxicity of crude oil, and it is worth noting that formation of damage in bivalve mollusks (except nuclear buds) can appear soon after oil spillage in the marine environment.

The genotoxicity and cytotoxicity of crude oil from the Statfjord B platform had earlier been proven in a 3-week treatment of turbot *Scophthalmus maximus* (Baršienė et al., 2006e; Bolognesi et al., 2006b) and in blue mussels (Baršienė, Andreikėnaitė, 2007). A significant elevation of MN levels after a 3-week treatment with 0.5 ppm of dispersed crude oil was found in turbot blood and kidney erythrocytes, together with elevation of nuclear buds and bi-nucleated cells in kidney erythrocytes (Baršienė et al., 2006e). A statistically significant induction of MN and fragmented-apoptotic cells was registered in mussels exposed to 0.5 ppm of crude oil or to spiked 0.5 ppm of the oil. A significant increase of nuclear buds was observed after exposure to spiked 0.5 ppm of crude oil (Baršienė, Andreikėnaitė, 2007). Time-related changes of MN and other nuclear abnormalities were observed in Atlantic cod liver erythrocytes after 3-, 14- and 24-day treatments with different concentrations of crude oil from the North Sea. The induction of MN was highest after 14 days and of nuclear buds, fragmented-apoptotic and bi-nucleated cells after a 24-day exposure to 0.25 ppm, to 1 ppm, or to 1 ppm spiked oil (our unpublished data).

Crude oil consists of different components such as various hydrocarbons, heavy metals, nitrogen-oxygen compounds. The content of components differs depending on oil processing sites (Wake, 2005). In order to assess the potential genotoxicity and cytotoxicity of oil from the Minija well (Lithuania), we performed a 10-day exposure of freshwater bivalve *Anodonta anatina* and perch *Perca fluviatilis* to 0.25 ppm, 0.5 ppm and 1 ppm of crude oil. Nuclear abnormalities were studied in bivalve gills and perch peripheral blood. The non-parametric Mann–Whitney U-test revealed the largest differences in MN induction among mussels, perch from the control groups and those treated with 0.5 ppm. Treatment with 0.25 ppm crude oil resulted in MN induction only in bivalves. The frequency of nuclear buds was not increased in the exposed groups of mussels and fish. Exposure of *A. anatina* and *P. fluviatilis* to 1 ppm of Lithuanian crude oil did not increase genotoxicity levels, but significantly elevated the induction of bi-nucleated cells in gills of bivalves and the incidence of fragmented-apoptotic cells in fish blood (Baršienė et al., 2006c). A species-specific pattern in genotoxicity response to crude oil exposure was shown in bivalve mollusks (Hamoutene et al., 2002) and in fish (Baršienė et al., 2006c). DNA damage in mussel *Mytilus galloprovincialis* significantly increased after a 12-day exposure to Arabian light crude oil, but did not induce damage in clams *Mya arenaria* (Hamoutene et al., 2002). These studies demonstrated a potential environmental genotoxicity resulting from different oil spills as well as pointed to interspecies sensitivity.

In the crude oil processed from the Statfjord B platform, polycyclic aromatic hydrocarbons (PAH) constitute about 1.5% of the total weight. Naphthalene and its derivatives prevailed in the crude oil and showed the highest bioavailability in fish (Sundt et al., 2006). A time-dependent ability of naphthalene and benzo[*a*]pyrene (concentrations 0.1, 0.3, 0.9, or 2.7 μM for both compounds) to induce micronuclei in mature erythrocytes after a short-term exposure (2, 4, 6 and 8 h) has been recorded in juvenile *Dicentrarchus labrax* fish. A significant induction of MN and other nuclear abnormalities was observed after 4-h of treatment with 0.3, 0.9 and 2.7 μM, upon a 6-h exposure to 0.9 and 2.7 μM, an 8-h exposure to 0.3, 0.9 and 2.7 μM of naphthalene (Gravato, Santos, 2002). Significantly increased levels of nuclear abnormalities were described in *D. labrax* erythrocytes after 2-h of exposure to 0.1, 0.9, and 2.7 μM benzo[*a*]pyrene (B[*a*]P), after 4-h of exposure to 0.1 and 0.9 μM. Interestingly, 6-h and 8-h exposures to low B[*a*]P concentrations (0.1 and 0.3 μM) caused the highest increase of nuclear abnormalities as compared to control (Gravato, Santos, 2002).

In eel *Anguilla anguilla*, treatment with 0.3, 0.9 and 2.7 μM of naphthalene induced micronuclei and other nuclear abnormalities (Teles et al., 2003). An increased frequency of MN was observed in mussels after 15-day exposure to 0.1 μg/L of phenanthrene (Koukouzika, Dmitriadis, 2008), in zebra mussel *Dreissena polymorpha* after 2-, 3- and 4-day treatments with different concentrations (2 μg/L and 10 μg/L) of B[*a*]P (Binelli et al., 2008).

Benzo[*a*]pyrene was classified as a progenotoxin which after metabolic activation becomes a very aggressive DNA-damaging agent (Johnson, 1992). Genotoxic effects of B[*a*]P and dimethylbenz[*a*]antracene had been demonstrated earlier in gills and hemolymph of marine molluscs (Burgeot et al., 1995; Bolognesi et al., 1996; Venier et al., 1997; Siu et al., 2004). Comet and MN assays have indicated clear dose- and time-dependent responses to B[*a*]P exposure in *Mytilidae* bivalve *Perna viridis* (Siu et al., 2004). DNA strand breaks were significantly different from control levels from 1- to 6-day treatment and then a gradual decrease to control levels upon a 20-day exposure in scallops *Chlamys farreri* treated with 0.5 and 3 μg/L B[*a*]P (Pan et al., 2008). Data were reported on the genotoxicity of 10 polycyclic aromatic hydrocarbons (anthracene, benz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, dibenz[*a,h*]anthracene, dibenz[*a,c*]anthracene, 3-methylcholanthrene, benzo[*a*]pyrene, benzo[*e*]pyrene, chrysene and pyrene) in mice skin cells, and it was pointed out that the genotoxicity of these compounds in general correlated with their carcinogenicity (Nishikawa et al., 2005).

The extensive development of offshore oil industry in the North Sea and arctic regions requires an integrated system of well-developed methods of estimating the ecological risk of oil contamination in marine ecosystems. However, there is a lack of validated tests, simple, cost-effective screening methods, which could be applied effectively, taking into consideration environmentally realistic routes of contamination in oil platform areas. The present study results have demonstrated the genotoxic and cytotoxic potentials of the North Sea crude oil in mussels, a cosmopolitan and ecologically relevant organism. Validation of the micronuclei test and the approval of other nuclear abnormalities as biomarkers of environmental genotoxicity and cytotoxicity were performed using different marine fish and mussel species in laboratory treatments with crude oil from different platforms, with a mixture of alkylphenols, PAHs, wastewater, flame retardants or endocrine disruptor concentrations. Contaminant-species-tissue-exposure time-specific responses have been described (Baršienė et al., 2005, 2006a, 2006c, 2006d; Andreikėnaitė et al., 2007; Baršienė, Andreikėnaitė, 2007). Thus, the developed approach and the collected information should also help to fill gaps in our understanding of the ecological significance of crude oil pollution in marine ecosystems. The endpoints used in our study may serve as early warning signs offering an evidence of genotoxicity risks in wildlife species and for describing the cytological mechanisms of crude oil toxicity. This biomarker system could be successfully used for the assessment of background levels before offshore oil installations, as well as within the frames of environmental quality monitoring. Laboratory-controlled experiments, active monitoring approaches, and *in situ* assessment of DNA damage in various tissues, especially in gonads of target species, will help to achieve a substantial progress in the assessment of early responses as well as short- or long-term adaptations to chronic pollution originating from petroleum installations.

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References

1. Andreikėnaitė L., Baršienė J., Vosylienė M. Z. 2007. Studies of micronuclei and other nuclear abnormalities in blood of rainbow trout (*Oncorhynchus mykiss*) treated with heavy metal mixture and road maintenance salts. *Acta Zoologica Lituanica*. Vol. 17. P. 213–219.
2. Baršienė J., Baršytė Lovejoy D. 2000. Environmental genotoxicity in Klaipėda port area. *International Review of Hydrobiology*. Vol. 85. P. 663–672.
3. Baršienė J. 2002. Genotoxic impacts in Klaipėda marine Port and Būtingė oil terminal areas (Baltic Sea). *Marine Environmental Research*. Vol. 54. P. 475–479. doi:10.1016/S0141-1136(02)00160-5
4. Baršienė J., Lazutka J., Šyvokienė J., Dedonytė V., Rybakovas A., Bjornstad A., Andersen O. K. 2004. Analysis of micronuclei in blue mussels and fish from the Baltic and the North Seas. *Environmental Toxicology.* Vol. 19. P. 365–371. doi:10.1002/tox.20031
5. Baršienė J., Dedonytė V., Rybakovas A., Broeg K., Forlin L., Gercken J., Kopecka J., Balk L. 2005. Environmental mutagenesis in different zones of the Baltic Sea. *Acta Zoologica Lituanica*. Vol. 15. P. 90–95.
6. Baršienė J., Rybakovas A. 2006. Cytogenetic and cytotoxic effects in gill cells of the blue mussel *Mytilus edulis* from the Baltic coast and after 1–3-day maintenance in laboratory. *Acta Zoologica Lituanica*. Vol. 16. P. 191–197.
7. Baršienė J., Schiedek D., Rybakovas A., Šyvokienė J., Kopecka J., Forlin L. 2006a. Cytogenetic and cytotoxic effects in gill cells of the blue mussel *Mytilus* spp. from different zones of the Baltic Sea. *Marine Pollution Bulletin*. Vol. 53. P. 469–478. doi:10.1016/j.marpolbul.2005.11.015
8. Baršienė J., Lehtonen K., Koehler A., Broeg K., Vourinen P. J., Lang T., Pempkowiak J., Šyvokienė J., Dedonytė V., Rybakovas A., Repečka R., Vountisjarvi H., Kopecka J. 2006b. Biomarker responses in flounder (*Platichthys flesus*) and

mussel (*Mytilus edulis*) in the Klaipėda–Būtingė area (Baltic Sea). *Marine Pollution Bulletin*. Vol. 53. P. 422–436. doi:10.1016/j.marpolbul.2006.03.009

9. Baršienė J., Andreikėnaitė L., Rybakovas A. 2006c. Cytogenetic damage in perch (*Perca fluviatilis* L.) and Duck mussel (*Anodonta anatina* L.) exposed to crude oil. *Ekologija*. Vol. 1. P. 25–31.
10. Baršienė J., Šyvokienė J., Bjornstad A. 2006d. Induction of micronuclei and other nuclear abnormalities in mussels exposed to bisphenol A, diallyl phthalate and tetrabromodiphenyl ether-47. *Aquatic Toxicology*. Vol. 78S. P. S105–S108.
11. Baršienė J., Dedonytė V., Rybakovas A., Andreikėnaitė L., Andersen O. K. 2006e. Investigation of micronuclei and other nuclear abnormalities in peripheral blood and kidney of marine fish treated with crude oil. *Aquatic Toxicology*. Vol. 78S. P. S99–S104. doi:10.1016/j.aquatox.2006.02.022
12. Baršienė J., Andreikėnaitė L. 2007. Induction of micronuclei and other nuclear abnormalities in blue mussels exposed to crude oil from the North Sea. *Ekologija*. Vol. 53. No. 3. P. 9–15.
13. Baršienė J., Andreikėnaitė L., Garnaga G., Rybakovas A. 2008. Genotoxic and cytotoxic effects in bivalve mollusks *Macoma balthica* and *Mytilus edulis* from the Baltic Sea. *Ekologija*. Vol. 54. No. 1. P. 44–50.
14. Binelli A., Riva C., Cogni D., Provini A. 2008. Assessment of the genotoxic potential of benzo(a)pyrene and *pp*'-dichlorodiphenyldichloroethylene in Zebra mussel (*Dreissena polymorpha*). *Mutation Research*. Vol. 649. P. 135–145. doi:10.1016/j.mrgentox.2007.08.011
15. Bocchetti R., Fattorini D., Pisanelli B., Macchia S., Oliviero L., Pilato F., Pellegrini D., Regoli F. 2008. Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis,* to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas. *Aquatic Toxicology*. Vol. 89. P. 257–266. doi:10.1016/j.aquatox.2008.07.011
16. Bolognesi C., Rabboni R., Roggieri P. 1996. Genotoxicity biomarkers in *M. galloprovincialis* as indicators of marine pollutants. *Comparison Biochemical Physiology*. Vol. 113C. P. 319–323.
17. Bolognesi C., Perrone E., Roggieri P., Sciutto A. 2006a. Bioindicators in monitoring long term genotoxic impact of oil spill: Haven case study. *Marine Environmental Research*. Vol. 62. P. S287–S291. doi:10.1016/j.marenvres.2006.04.047
18. Bolognesi C., Perrone E., Roggieri P., Pampanin D. M., Sciutto A. 2006b. Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. *Aquatic Toxicology*. Vol. 78S. P. S93–S98. doi:10.1016/j.aquatox.2006.02.015
19. Burgeot T., His E., Galgani F. 1995. The micronucleus assay in *Crassostrea gigas* for the detection of seawater genotoxicity. *Mutation Research*. Vol. 343. P. 125–140.
20. Carvalho Pinto-Silva C. R., Creppy E., Matias W. 2005. Micronucleus test in mussels *Perna perna* fed with the toxic dinoflagellate *Prorocentrum lima*. *Archives of Toxicology*. Vol. 79. P. 422–426.
21. Dolcetti L., Venier P. 2002. Susceptibility to genetic damage and cell types in Mediterranean mussels. *Marine Environmental Research*. Vol. 54. P. 487–491.
22. Dyrynda E. A., Law R. J., Dyrynda P. E. J. 1997. Modulations in cell-mediated immunity of *Mytilus edulis* following the "Sea Empress" oil spill. *Journal of the Marine Biological Association. UK*. Vol. 77. P. 281–284.
23. Fenech M., Chang W. P., Kirsch-Volders M., Holland N., Bonassi S., Zeiger E. 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutation Research*. Vol. 534. P. 65–75. doi:10.1016/S1383-5718(02)00249-8
24. Francioni E., Wagener A de L. R., Scofield A de L., Depledge M. H., Cavalier B., Sette C. B., Carvalhosa L., Lozinsky C., Mariath R. 2007. Polycyclic aromatic hydrocarbon in inter-tidal mussel *Perna perna*: Space-time observations, source investigation and genotoxicity. *Science of the Total Environment*. Vol. 372. P. 515–531. doi:10.1016/j.scitotenv.2006.08.046
25. Gravato C., Santos M. A. 2002. Juvenile sea bass liver P450, EROD induction, and erythrocytic genotoxic responses to PAH and PAH-like compounds. *Ecotoxicology and Environmental Safety*. Vol. 51. P. 115–127.
26. Hamoutene D., Payne J. F., Rahimtula A., Lee K. 2002. Use of the Comet assay to assess DNA damage in hemocytes and digestive gland cells of mussels and clams exposed to water contaminated with petroleum hydrocarbons. *Marine Environmental Research*. Vol. 54. P. 471–474. doi:10.1016/S0141-1136(02)00162-9
27. Harvey J. S., Lyons B., Page T. S., Stewart C., Parry J. M. 1999. An assessment of the genotoxic impact of the Sea Empress oil spill by the measurement of DNA adduct levels in selected invertebrate and vertebrate species. *Mutation Research*. Vol. 441. P. 103–114. doi:10.1016/S1383-5718(99)00037-6
28. Heddle J. A., Cimino M. C., Hayashi M., Romagna F., Shelby M. D., Tucker J. D., Vanparys Ph., MacGregor J. T. 1991. Micronuclei as an index of cytogenetic damage: past, present, and future. *Environmental and Molecular Mutagenesis*. Vol. 18. P. 277–291. doi:10.1002/em.2850180414
29. Hylland K., Tollefsen K. E., Ruus A., Jonsson G., Sundt R. C., Sanni S., Utvik T. I. R., Johnsen I., Pinturier L., Balk L., Baršienė J., Marigomez I., Feist S. W., Borseth J. F. 2008. Water column monitoring near oil installations in the North Sea 2001–2004. *Marine Pollution Bulletin*. Vol. 56. P. 414–429. doi:10.1016/j.marpolbul.2007.11.004
30. Izquierdo J. I., Machado G., Ayllon F., d'Amico V. L., Bala L. O., Vallarino E., Elias R., Garcia-Vazquez E. 2003. Assessing pollution in coastal ecosystems: a preliminary survey using the micronucleus test in the *Mytilus edulis*. *Ecotoxicology and Environmental Safety*. Vol. 55. P. 24–29. doi:10.1016/S0147-6513(02)00041-6
31. Jha A. N. 2008. Ecotoxicological applications and significance of the comet assay. *Mutagenesis Advance Access*. Vol. 1. P. 1–15.
32. Johnson B. T. 1992. Potential genotoxicity of sediments from the Great Lakes. Environmental Toxicology. *Water Quality International*. Vol. 7. P. 373–390.

33. Klobučar G. I. V., Štambuk A., Hylland K., Pavlica M. 2008. Detection of DNA damage in haemocytes of *Mytilus galloprovincialis* in the coastal ecosystems of Kaštela and Trogir bays, Croatia. *Sciences of the Total Environment*. Vol. 405. P. 330–337. doi:10.1016/j.scitotenv.2008.05.015
34. Koukouzika N., Dimitriadis V. K. 2008. Aspects of the usefulness of five marine pollution biomarkers, with emphasis on MN and lipid content. *Marine Pollution Bulletin*. Vol. 56. P. 941–949. doi:10.1016/j.marpolbul.2008.01.043
35. Lindberg H. K., Wang X., Järventaus H., Falck G. C. M., Norppa H., Fenech M. 2007. Origin of nuclear buds and micronuclei in normal and folate-deprived human lymphocytes. *Mutation Research*. Vol. 617. P. 33–45. doi:10.1016/j.mrfmmm.2006.12.002
36. Magni P., de Falco G., Falugi C., Franzoni M., Monteverde M., Perrone E., Sigro M., Bolognesi C. 2006. Genotoxicity biomarkers and acetylcholinesterase activity in natural populations of *Mytilus galloprovincialis* along a pollution gradient in the Gulf of Oristano (Sardinia, western Mediterranean). *Environmental Pollution*. Vol. 142. P. 65–72. doi:10.1016/j.envpol.2005.09.018
37. Mičic M., Bihari N., Jaksic Z., Muller E. G., Batel R. 2002. DNA damage and apoptosis in the mussel *Mytilus galloprovincialis. Marine Environmental Research*. Vol. 53. P. 243–262.
38. Nishikawa T., Nakamura T., Fukushima A., Takagi Y. 2005. Further evaluation of the skin micronucleus test: Results obtained using 10 polycyclic aromatic hydrocarbons. *Mutation Research*. Vol. 588. P. 56–63. doi:10.1016/j.mrgentox.2005.09.004
39. Ohe T., Watanabe T., Wakabayashi K. 2004. Mutagens in surface waters: a review. *Mutation Research*. Vol. 567. P. 109–149. doi:10.1016/j.mrrev.2004.08.003
40. Pan L., Miao J., Wang J., Liu J. 2008. AHH activity, tissue dose and DNA damage in different tissues of the scallop *Chlamys farreri* exposed to benzo[*a*]pyrene. *Environmental Pollution*. Vol. 153. P. 192–198. doi:10.1016/j.envpol.2007.07.022
41. Parry J. M., Harvey J. S., Lyons B. P. 1997. The application of genetic toxicology in the analysis of the consequences of a major marine pollution incident. *Mutation Research*. Vol. 379. Issue 1. P. S91.
42. Røe T. U. 1999. Chemical characterisation of produced water from four offshore oil production platforms in the North Sea. *Chemosphere*. Vol. 39. P. 2593–2606.
43. Siu W. H. L., Cao J., Jack R. W., Wu R. S. S., Richardson B. J., Xu L., Lam P. K. S. 2004. Application of the comet and micronucleus assays to the detection of B[*a*]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). *Aquatic Toxicology*. Vol. 66. P. 381–392. doi:10.1016/j.aquatox.2003.10.006
44. Sundt R. C., Pampanin D. M., Larsen B. K., Brede C., Herzke D., Bjornstad A., Andersen O. K. 2006. The BEEP Stavanger workshop: mesocosm exposures. *Aquatic Toxicology*. Vol. 78S. P. S5–S12. doi:10.1016/j.aquatox.2006.02.012
45. Teles M., Pacheco M., Santos M. A. 2003. *Anguilla anguilla* L. liver ethoxyresorufin O-deethylation, Glutathione S-transferase, erythrocytic nuclear abnormalities, and endocrine responses to naphthalene and β-naphthoflavone. *Ecotoxicology and Environmental Safety*. Vol. 55. P. 98–107. doi:10.1016/S0147-6513(02)00134-3
46. Venier P., Maron S., Canova S. 1997. Detection of micronuclei in gill cells and haemocytes of mussels exposed to benzo[*a*]pyrene. *Mutation Research*. Vol. 390. P. 33–44.
47. Venier P., Zampieron C. 2005. Evidence of genetic damage in grass gobies and mussels from the Venice lagoon. *Environment International*. Vol. 31. P. 1053–1064. doi:10.1016/j.envint.2005.05.016
48. Wake H. 2005. Oil refineries: a review of their ecological impacts on the aquatic environment. *Estuarine, Coastal and Shelf Science*. Vol. 62. P. 131–140. doi:10.1016/j.ecss.2004.08.013
49. Woodhead R. J., Law R. J., Matthiessen P. 1999. Polycyclic aromatic hydrocarbons in surface sediments around England and Wales, and their possible biological significance. *Marine Pollution Bulletin*. Vol. 9. P. 773–790.

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BRANDUOLIO PAŽAIDŲ SUSIFORMAVIMAS MIDIJŲ *MYTILUS EDULIS* ŽIAUNŲ LĄSTELĖSE PO 1, 2, 4 IR 8 PARŲ POVEIKIO ŠIAURĖS JŪROJE IŠGAUNAMA ŽALIAVINE NAFTA

S a n t r a u k a

Genotoksinis ir citotoksinis Statfjord B naftos platformos (Šiaurės jūra) žaliavinės naftos poveikis laike įvertintas midijų *Mytilus edulis* žiaunų ląstelėse. Moliuskai 1, 2, 4 ir 8 paras veikti 0,5 ppm Statfjord B gręžinio nafta. Genotoksinis teršalų poveikis buvo vertinamas pagal mikrobranduolių (MB) ir branduolio pumpurų (BP) susiformavimą, o citotoksinis – pagal dvibranduolių (DB) ir fragmentuotų-apoptozinių (FA) ląstelių dažnį. Po 1, 2, 4 ir 8 parų ekspozicijos nustatytas laipsniškas, atsižvelgus į poveikio trukmę, MB ir DB parametrų didėjimas: kontrolinė gr.< po 1 paros < po 2 parų < po 4 parų < po 8 parų. Po 8 parų nustatytas MB kiekis buvo 2,4 karto, FA – 2 kartus, o DB – 3 kartus didesnis nei kontrolėje. Statistiškai patikimi MB dažnių skirtumai rasti tarp kontrolinės bei pirmos (P = 0,0185), antros (P = 0,0039), ketvirtos (P = 0,0068) ir aštuntos paros (P < 0,0001) *M. edulis* grupių. Statistiškai patikimų branduolio pumpurų skirtumų nerasta, tuo tarpu po 4 parų nustatyta statistiškai patikima FA ląstelių indukcija (P = 0,0115), o po 8 parų ekspozicijos – DB (P = 0,0232).

Raktažodžiai: mikrobranduoliai, branduolio pažaidos, žaliavinė nafta, midijos