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Short communication

Ecological study of gas fields in the northern Adriatic

13. Toxicological tests on organisms

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Wishing to have an insight into the level of exposure of sea organisms to noxious materia, toxicological tests have been carried out on blue mussels Mytilus galloprovincialis. *No mutagens have been found.*

13.1. INTRODUCTION

Tests indicating the presence of cancerogens have been performed as to provide a better insight into the exposure of marine organisms to noxious materia. The chosen organism was the mussel, that was recommended for such tests by UNEP.

13.2. MATERIAL AND METHODS

Toxicological tests performed on mussels sampled March 14, 1986 on the PANON platform in the zone of the IVANA field in the Štinjan cove, and on control mussels sampled March 25, 1986 in the Limski Channel. Preliminary tests performed on mussels sampled on December 12, 1985 on the PANON Platform (Ivana field) and in the Štinjan cove, that is on control mussels from the Šibenik mariculture sampled on December 12, 1985.

For the purpose of extraction, xenobiotics were extracted from the homogenate of the soft part of the mussel with 1.5 volume (v/v) ethyl acetate: acetone (2:1) (BAIRD et al., 1980). The dry remainder of the ethyl acetate phase is dissolved in dimethyl sulphoxide (DMSO) (for the Ames test) or in corn oil (for the induct test).

The Ames test to mutagenicity (carcinogenicity) test is performed according to the MARON and AMES method 1983) with the tester strain of Salmonella typhimurium TA 98 (courtesy of dr B.N. Ames, Univ. of California, Berkeley, Dept. of Biochemistry). As the activating factor in the S9 mix, the post-mitochondrial fraction of carp liver was used (1+) inducted by intraperitoneal (i.p.) injection 3-methylcholanthrene (3-MC) (50 mg/kg) (PROTIĆ-SABLJIĆ and KURELEC, 1983). As the positive control, the benzo(a)pyrene (BaP) was used as an indirect cancerogen. The mutagenicity/carcinogenicity was tested in aliquots of the extract equivalent to 0.5, 1 and 5 g. of the wet weight of the mussel tissue. All tests were performed in duplicate (2 plates per sample).

In order to measure the mixed function oxidases - the induct test (MFO-induct test) for xenobiotics presence, the amount of xenobiotics in the sample was measured by the method of increase monitoring (induction) of the basal activity of the benzo(a)pyrene monooxygenase (BaPMO) in the carp liver after i.p. injection of aliquotes of the extract of tissue dissolveded into $0.1cm^3$ of oil (KURELEC and coll., 1982; PROTIĆ-SABLJIĆ and KURELEC, 1983). Each sample was applied

in the equivalent of 1g of wet weight in 4 fishes. The group of 4 fishes after i.p. application of 50 mg/kg 3-MC served as a positive control. Forty-eight hours after treatment, the fish were decapitated and in the postmitochondrial fraction of liver homogenate the activity of the BaMPO was determined by the NEBERT and GELBOIN method (1968). The BaMPO activity expressed in pmols of the occurred BaP hydroxide per min per mg of protein (determined according to the LOWRY and coll. method, 1951). The quantity of inducted xenobiotics in the extract was expressed as the equivalent of the 3-MC quantity (in mg) that, applied i.p., causes equal induction of BaMPO activity in carp.

*Table 13.1. Quantities of Direct and Indirect Mutagens in Mussel Extracts**

Location	Quantity	Number of TA 98 revertans	
	g/plat	no activation	with activation
Panon	0.5	27.30	30.33 (34.39)*
	1	31.26	27.23 (38.50)
	5	25.25	26.29 (29.49)
Štinjan	0.5	28.31	28.25 (48.50)
	1	30.27	33.28 (59.36)
	5	20.24	24.27 (toksično)
Lim	0.5	22.37	36.34 (54.45)**
	1	24.26	47.37 (44.40)
	5		(45.42)
Control		32.24	26.27 (37.45)
DMSO	100 ul		24.27 (47.39)
BaP	100 ug	31.28	132.139 (64.80)

* - results of the preliminary Ames test on samples dated December 7, 1985 are in brackets

** - controls in brackets refer to samples from the Šibenik mariculture dated December 12, 1985

*Table 13. 2. MFO-induct test of Mussel Extracts**

Location	Activity BaPMO	3-MC equivalent (mg)
Panon	4.96 ± 1.33 (4.46 ± 1.69)**	20.1
Štinjan	3.05 ± 0.45 (3.45 ± 0.52)	12.2
Lim	3.34 ± 0.29	13.6
Control		
Not treated	2.01 ± 0.22	7.5
3-MC, 50mg/kg	11.79 ± 1.15	50.0

* - each sample was tested on 4 fish with quantities that are equivalent to the extract from 1 g of mussel tissue. BaMPO values represent the mean value ± standard deviation. The quantity of inducing xenobiotics in the extract is expressed as the equvivalent to the 3-MC amount (in mg) that in the carp causes equal induction of BaMPO activity

** - in brackets are values obtained in preliminary tests of extracts mussels sampled on December 7, 1985

13.3. TEST RESULTS

13.3.1. Test on the Presence of Mutagens/Cancerogens

Table 13.1. brings Ames test results on mussels extracts. From the results it is evident that mussel extracts from the PANONA do not differ from the mussels' extracts from the Štinjan cove, or controlled mussels from the Limski Channel. Neither extract tested in quantities that were equivalent to 0.5, 1 and 5 grams of mussel tissue did not contain neither direct (without activation) nor indirect (with activation) mutagens. Indirect (and most probably direct as well) mutagens are also not contained in extracts of mussels collected at PANON and in Štinjan on December 7, 1985.

13.3.2. MFO-induct test on Xenobiotics

Table 13.2. brings induct test results. It is evident that mussel extracts from the Limski Channel and Štinjan increase the BaMPO basal activity whose value is expressed as an induction equivalent caused with 7.5 mg 3-MC, up to the value of 13 mg 3-MC equivalent. Both samples from the PANON contain xenobiotics that induct BaMPO up to the value of 17.6 i.e. 20.1 3-MC equivalent, or 7.1 mg 3-MC equivalents per 1 gram of net tissue.

13.4. CONCLUSION

No cancerogens were found in examined shellfish, and the IVANA and IKA field as well as the Štinjan cove may be considered inocuous from that point of view.

13.5. REFERENCES

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Ekološka studija plinskih polja u sjevernom Jadranu

Toksikološki testovi na organizmima

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KRATKI SADRŽAJ

Kako bi se odredila razina izloženosti morskih organizama toksičnim tvarima izvršeni su toksikološki testovi na dagnji. U ispitanim školjkama nisu nađene kancerogene tvari pa se područje polja IVANA kao i uvala Štinjan može se smatrati još neugroženom s tog aspekta.