Effects of an Organophosphorus Pesticide on the behaviour of *Monodonta turbinata* (Marine Gastropod, Trochid)

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Organophosphorus pesticides have been increasingly used in agriculture because of their relative instability in the environment. These pesticides and potential marine contaminants interfere with neurophysiological activities and therefore it is possible that they also interfere with the normal behavioural and locomotory activities of exposed marine organisms. A series of investigations are being undertaken to study the effects of such contaminants on a range of marine invertebrates. In the present paper, some results are reported on the effects of Malathion at a relatively low concentration of 0.1 ppm, on the immersion-emersion activity of a locally abundant littoral trochid, *Monodonta turbinata*. This trochid, normally settles near the water surface, periodically moving up and down through this surface. This immersion-emersion activity is significant to the respiratory activities of *Monodonta* species and was found to be dependent on ambient temperature (MICALLEF, 1971).

This behavioural activity has been studied in the laboratory by means of an aktograph, first described by MICALLEF (1971). All experiments were carried out in a constant temperature room at constant temperatures ranging from 18 to 23 in different runs and with diffuse overhead artificial lighting switched on for 12 hours daily. Animals and sea water (salinity 34.4-36.8 ppt) were collected from unpolluted rocky shores on the northwest coast of Malta. Animals were acclimated to laboratory conditions for at least 24 hours and then each was introduced in an aktograph holding 100 ml of unfiltered seawater. This aktograph, which has been described elsewhere (SALIBA and VELLA, 1977 ; AXIAK and SALIBA, 1982) is capable of recording any immersion or emersion of an individual trochid, by a movement of a lever which leaves a 24 hour trace on a revolving drum. In the series of experiments here described, Malathion was first dissolved in acetone and then dosed in the exposed aktographs to obtain a nominal concentration of 0.1 ppm. Each exposure experiment lasted for 96 hours with test mixtures (unaerated) being renewed every 24 hours. Detailed chemical analysis of the test mixtures over the exposure period, will be reported elsewhere. Control aktograph were dosed with the same amount of acetone as exposure aktographs. A total of 21, 96-hour exposure experiments and 21 96-hour controls were carried out during the period January-April, 1990.

Table 1 shows the number of complete immersions and emersions, as well as the time spend immersed, emersed and at interphase for individual animals over a 24 hour period (activities during the light and dark periods being shown separately). Means and coefficient of variations (cv) for each parameter are included. 76, 24 hour traces and 64, 24 hour traces were analysed for exposed and control animals.

These results indicate that both the control and the exposed animals are more active across the water interphase during the day then during the night. During the day, the numbers are complete immersions and emersions are higher than during the night. Moreover, the animals spend more time immersed or in the water interphase.

Table 1

	Treatment : Day Period :	Control Light	Control Dark	Exposed Light	Exposed Dark
No of Immersions	means	5.0	1.8	1.6	0.4
	cv	6.44	5.16	3.69	2.42
No of Emersions	means	5.8	1.9	2.6	0.3
	cv	5.21	4.94	2.45	2.58
% time immersed	means	7.1	1.4	6.7	3.2
	cv	6.78	2.99	19.62	41.72
% time emersed	means	37.3	49.0	40.6	47.0
	cv	3.34	0.31	3.60	2.85
% time at interphase	means	4.6	0.7	1.3	0.5
	cv	11.22	3.55	4.03	8.10

These effects were found to be statistically significant ($P<0.01$) when means of the various parameters were compared by t-test as well as the Mann-Whitney U test (non-parametric test). Moreover, as is expected in such behavioural studies, a wide range of variability is exhibited by the various parameters monitoring both in the control and exposed animals. Animals exposed to a nominal concentration of 0.1ppm Malathion were more active across the water interphase and spent more time out of the water rather than at the interphase or immersed. These effects were statistically significant at the 0.05 P level as determined by t-tests on the respective means. This effect of Malathion at this low concentration may be considered as an adaptative change in the normal behaviour of this trochid which would enable it to come in contact with the contaminated water less frequently. This would presumably however affect the respiratory activities of the animal. Alternatively, this change behaviour could be interpreted as a manifestation of neurophysiological toxicity. Exposure to higher concentrations of Malathion (0.5 ppm) in fact induced the animals to enter into an inactive state and remain at the bottom of the aktograph throughout most of the exposure period. This activity of *Monodonata* is also known to be affected by other pollutants (SALIBA and VELLA, 1977 ; AXIAK and SALIBA, 1982).

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Diagnosis of factors influencing in vitro oil toxicity assessment

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Crude oils as all purely organic pollutants in the marine environment (as well as during *in vitro* bioassay experiments) are naturally subjected to many quantitative and qualitative modifications. By consequence, test organisms should have variable responses depending upon the instance composition and concentration of oil in their media.

From methodological point of view, the characteristics of the followed method for preparing the oily working medium play an essential part in the magnitude of constitutional variation of oil during the bioassay duration. In fact, any slight variation in the preparative steps of oily working medium (ratio of oil to water, mixing mean, power or duration, etc...) leads to significant variation in the potential of applied initial oil concentration and composition. Unsufficient attention is paid for this primordial aspect in the MAP technical report, devoted for oil ecotoxicological research (UNEP/FAO, 1987).

The factors and variables influencing the evaluation of oil toxicity on the marine organisms could be divides into four groups : form of oil in test medium, test medium preparative steps, water quality and test organism characteristics. Among these groups of factors, the present work includes results concerning the effect of variations in oily test medium preparation steps on the applied oil concentration in the bioassay experiments. Also, the relationship between initial preparated oil concentration in test medium, toxic effect (fish mortality) and instantaneous oil concentration in the medium (measured at the time of bioassay when a certain toxic effect is observed) is discussed.

The materials of this work are two types of arabian crude oils (light and heavy), 3 chemical dispersants (Finasol-2, -5 and -7) and one species of fish (*Lebistes reticulatus*) as test organisms for *in vitro* bioassay experiments. Throughout series of tests for preparing oily test media, ratios of oil : water or oil : dispersant : water, mechanically shaking power and duration and stabilization time of the produced mixtures are systematically varied. For each test, dissolved and dispersed oil residues are measured (IOC/WMO, 1976). In another series of bioassay experiments (Fig. 1), in which dispersed oil is used, lethal effect is followed for 5 days in comparison with the initial and instantaneous oil concentration in the medium.

From one side, the results showed the significant primordial role of any slight variation in the characteristics of even one of the preparative steps of used oily medium in bioassay tests. From the other side, effect of oil is related more to its instantaneous concentration in the medium than to the initial applied one. These results are supporting the necessity of the need of a standard method for *in vitro* oil ecotoxicological studies. Without such method, a convincing and precise overall picture for the real oil pollution effects in the marine environment could never be attained (PATIN, 1982).

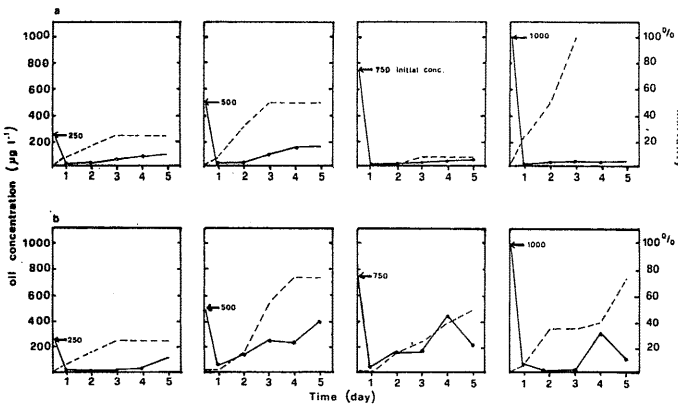

FIGURE 1 : Variations in fish-test mortality (—) in relation to variation in instantaneous dispersed light (a) and heavy arabian (b) oil concentration (- - -) with time using Finasol-7.

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