

Valve movement behaviour and byssal formation of the mussel, *Mytilus edulis* in relation to environmental toxins

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ABSTRACT

The present investigation was undertaken to determine behavioural responses of mussel, *Mytilus edulis*, to sub-lethal concentrations of lindane and atrazine. Behaviour effects that have been studied in mussels were byssus formation, valve movement and valve gape. Lindane was more toxic, causing a 46% reduction of byssal formation, compared to control, after 7 days exposure to 0.9 mg/l. However, atrazine caused 50% reduction of byssal formation after 14 days exposure to 3.585 mg/l atrazine. The sensitivity of the tests increased with increasing the exposure time. Measurements of valve movement and gape showed that increasing exposure and accumulation of lindane increased the proportion of time spent resting (a closed to open ratio of 1.31 at 56 days, compared to a value of 0.05 in the controls). On the other hand, with atrazine the valves did not shut completely (valve closed to open time ratio of 0.3 at 56 days compared with a control value of 0.08) but were observed to have a smaller gape (average gape at 56 days 8.7 mm) than control mussels (average gape at 56 days 25.5 mm). The data of valve movement indicate that the rest period has increased by increasing the concentration and the time of exposure to lindane. Byssus formation in *M. edulis* was progressively reduced with time of exposure to the 1/2 LC₅₀ of the two pesticides. The byssogenesis test was proved to be a sensitive test in mussels and is suggested as a convenient and rapid technique for bioassay of potential pollutants.

KEYWORDS: Mussels, valve movement, byssal formation, lindane and atrazine.

INTRODUCTION

Insecticides cause serious ecotoxicological problems mainly due to their persistence and high toxicity. The use of lindane (isomer gamma hexachlorocyclohexane, γ -HCH) is nowadays prohibited in most countries, but this organochlorine persists in soils and may reach the marine environment through erosion processes (Hamza-Chaffia *et al.* 1998). It is used as a foliar spray, in soil applications, as a seed treatment, and in baits for rodent control (Clark 1989). The herbicide, atrazine is used to control broadleaf weeds in many crops including maize, pineapples, sorghum, sugarcane, in apple and pear plants older than 4 year of age (Marchini *et al.* 1988). Aquatic herbicides have been widely used to control undesirable aquatic weeds in sites such as fish farms and rice fields (Hussein *et al.* 1996). Both atrazine and γ -HCH were chosen for study, because of their priority status as environmental contaminants. Lindane has acute toxicity values in the literature for a variety of aquatic organisms, while very few data is so far available for the species of mussels tested in this study.

Marine mussels are proving to be versatile biomonitors of pollution. They accumulate heavy metals and other substances from the environment indicating both the occurrence and level of a given pollutant in water and/or sediment. Recording the activity of mussels (opening and closing of the valves) is used for ecotoxicological testing of chemicals under laboratory conditions (Salánki 1979; Borcherdig & Wolf 2001). The blue mussel is a marine bivalve which lives attached to the rocks or piers by means of byssus threads. The attachment of marine

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species to hard substrata has long been of biological interest (Roberts 1975; Allen *et al.* 1976) and is achieved in mussels by means of protein threads (the byssus) secreted by pedal glands. Both valve movement and byssal formation reveal a great deal of information, concerning the physiological state of the bivalve. The production of byssus threads has been noticed to be affected by a range of toxicants (Roberts 1975) and therefore, it has been used as an indicator of exposure to pollutants.

The present investigation was carried out to determine behavioural responses of the mussel, *Mytilus edulis*, to sub-lethal concentrations of lindane and atrazine. Byssus formation, valve movement and valve gape were investigated, since behaviour represents an integrated response, corresponding to complex biochemical and physiological functions.

MATERIALS AND METHODS

Collection and maintenance of mussels (*Mytilus edulis*): Mussels 50-60 mm were collected from South Parade pier on the shore of Southsea, Portsmouth, UK. They were acclimatized to laboratory conditions for 2 weeks in tanks prior to experimentation. The tanks were supplied with a continuous flow of sea water from Langstone Harbour. The temperature varied depending on the time of the year between 8 °C and 20 °C, and salinity was 34 ‰. A natural photoperiod was maintained. The mussels were given no food other than that occurring naturally in water surrounding them. At the start of the experiments, they were transferred to a test cold room and again were given no additional food.

Chemicals: Gamma-hexachlorocyclohexane (γ -HCH) purity 99% was obtained from Aldrich, catalogue no. 23,339-0. Stock solutions were prepared in HPLC grade acetone (Fisher Laboratory Chemicals, A/0606/17), and stored at -20 °C. Stock solutions of atrazine (purity, 98.8 % from Chem Service) were prepared also by dissolving in HPLC grade acetone. 1/2 LC₅₀ of lindane (0.935 mg l⁻¹) and atrazine (3.585 mg l⁻¹) were used (El-Shenawy 1999).

Measurement of valve movement: Measurement of this behavioural activity response of mussels used the method of Salánki (1979) modified by Kramer *et al.* (1989) and Salánki (1991). It provides a potential early warning system used in mussel watch schemes. The control activity of mussels was recorded for 48 h before the application of toxicants. After this period, the desired amount of toxicant was added from a stock solution in acetone, to provide the appropriate concentration in the vessel. During the exposure period, the toxicant concentration was kept constant by restoring the concentration of the toxicant daily, when water was changed. The results were evaluated separately for each animal (n=13), the duration of active periods was measured and mean values for the control period and for the period following treatment were determined from the chart records. Activity in the presence of toxicants was expressed as a percentage of the control activity.

Valve gape

Valve gape of the mussels proved to be a more sensitive toxicological response, which could be used over a much wide range of lower concentrations than valve opening and closing. This toxicological response was measured up to 2 months of exposure to sublethal concentration (1/2 LC₅₀) of atrazine. Valve gape was measured as the shift of the lever on the transducer, and its values were used to calibrate the chart record. The width of valve gape was measured over a 24 h period at each sampling time.

Byssal production: Measurement of byssus formation has been used as indicator of toxicological effects on the activity of mussels. In this study, the method of Martin *et al.* (1975) was used to measure the byssal thread growth. The surface of the shell was cleaned of all fouling organisms and the emergent byssal threads were cut with scissors. Then, each mussel was kept in a separate tank and the test solution was changed daily. The number of

byssal thread produced by each individual was counted at a range of exposure times to the toxicant.

Statistical analysis: One way analysis of variance (ANOVA) and Student's t-test were used to test the significance of treatment effects and Duncan's multiple range test was used to test significance of differences between the effects of individual concentrations (Bailey 1981).

RESULTS

Evaluation of the mussel valve movement as a response to exposure to sub-lethal concentrations of pesticides: The effects of 60 days of exposure to $\frac{1}{2}$ LC₅₀ of lindane and atrazine on the duration of activity and rest period of the mussels are presented in figure 1. After a 24-h exposure to atrazine both the amplitude of shell valve opening and the rhythmic action have increased compared with the control activity (Fig. 2a) with no complete opening or closing (Fig. 2b). The effects of 7 days exposure to this sub-lethal concentration of atrazine are represented in Figure (2c), where a fast movement of the valves for extra pumping capacity is an attempt to refresh the mantle capacity. This characteristic pattern continued throughout the first 14 days of treatment. After a fortnight, the activity of the treated mussels had decreased to 84% compared with 92% for control mussels. There was no big difference between the activity of the mussels treated for 28 days and mussels exposed to atrazine for 56 days (78.4% and 74.6%, respectively) (Fig. 1). After 7 days of exposure, the frequency of valve openings and closings of the mussel ranged from 10 to 15 peaks h⁻¹ with high amplitude so that it was very difficult to calculate the overall duration of activity. However, recording the valve movement for the mussels exposed to $\frac{1}{2}$ LC₅₀ of atrazine over 56 days indicated that the rest period was increased and the amplitude of the contraction decreased with increasing exposure time (Fig. 3).

After 14 days of exposure to lindane the pattern of activity displayed a regular closing for an approximately constant period of time (Fig. 4a). At this stage the activity of the mussels had fallen to 75% compared to 95% for control (Table 2). It is clear that the activity decreased with increasing the exposure time to lindane, and activity had fallen to 43% at 56 days (Fig. 4b). The ratio of the time closed/time opened clearly indicated that under normal conditions mussels were closed for only a limited time with a maximum of 1.5-2h per day (Table 1). No significant change was observed in the ratio of closing and opening time of valve movement of mussels exposed to lindane for 14 days, but after 28 and 56 days of treatment, there was a significant difference in that ratio between control and treated mussels. The same ratio in *M. edulis* exposed to atrazine also had a strong effect (Table 2). Lindane had a far more marked effect on the activity of *M. edulis* than atrazine.

Fig.1: Effect of sub-lethal concentrations of lindane and atrazine on valve movement of *Mytilus edulis*

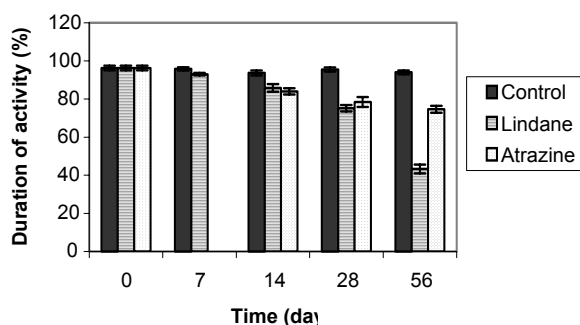


Table 1: Ratio of closing/opening time of the shell valves of *Mytilus edulis* exposed to a sub-lethal concentration of lindane. ^a The ratio of closing/opening time of valve movement based on 13 mussels; ^b Significantly different from the respective control (Student's t-test) $p < 0.001$.

Time (days)	Control ^a	lindane ^a
0	0.04	
7	0.05	0.07
14	0.06	0.17
28	0.05	0.33 ^b
56	0.05	1.31 ^b

Table 2: Effect of a sub-lethal concentration of atrazine on the ratio of closing/opening time of shell valves of the mussel, *Mytilus edulis*. ^a The ratio of closing/opening time of shell valves based on 10 mussels. ^b (---) It was not possible to measure the duration of the open and closed states because of the high frequency of opening and closing movements (Fig. 1b,1c). ^c Significantly different from the respective control (Student's t- test) $p < 0.001$.

Time (days)	Control ^a	atrazine ^a
0	0.04	
7	0.04	(---) ^b
14	0.08	0.2
28	0.04	0.3 ^c
56	0.08	0.3 ^c

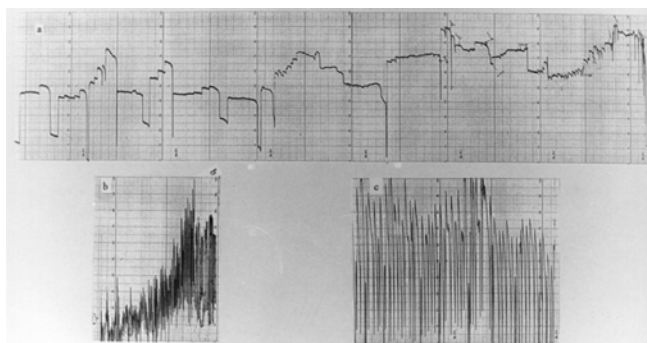


Figure 2: Examples of valve movement activity recorded (for 24h) for marine mussels exposed to atrazine (3.58 mg l^{-1}) for 7 days. a: control mussels, b: activity over the first few hours, and c: activity at the end of the 7th day. Downward deflections of the pen indicate valve closure, upward deflections, valve opening. The major division on the time axis of the chart represents one hour.

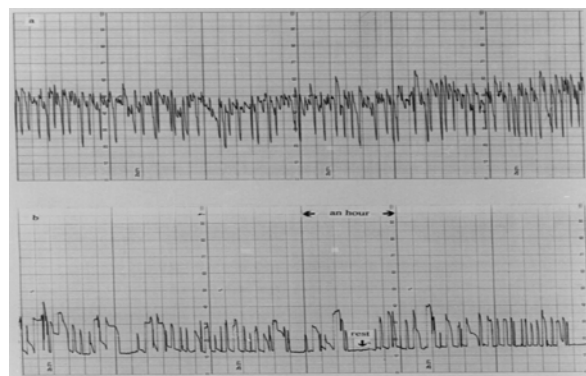


Figure 3: Examples of activity recorded from mussels treated with atrazine (3.58 mg l^{-1}). a: activity after 56 days exposure, and b: activity after 60 days exposure. In "b" the amplitude of the activity is small and the closed period elongated and repeated frequently. Downward deflections of the pen indicate valve closure, upward deflections, valve opening. The major division on the time axis of the chart represents one hour.



Figure 4: A characteristic trace of valve movement recorded for 24 h at each time point, a: 14 days mussels treated with lindane (0.9 mg l^{-1}) and b: activity after 56 days of treatment. Downward deflections of the pen indicate valve closure, upward deflections, valve opening. The major division on the time axis of the chart represents one hour.

Effect of a sub-lethal concentration of atrazine on the gape of the shell opening: Measurements of valve movement and gape showed that increasing exposure and accumulation of lindane increased the proportion of time spent resting (Table 1). But in experiments with atrazine the valves did not shut completely (Table 2) but had a narrower gape (average gape at 56 days 8.7 mm) than control mussels (average gape at 56 days 25.5 mm).

Although the present study indicated that there was no big difference in the duration of opening of mussels exposed to atrazine for 28 and 56 days, there appeared to be a difference in the extent of gape. There was an increase in the shell gape compared with the control values in the treated mussels after 7 and 14 days of exposure (Table 3). As exposure time

increased further, a decline in valve gape was observed and this was significantly lower in the treated mussels than controls after 28 and 56 days. Moreover, the percentage of time spent in the open state compared to the control level declined with elapsed time from the initially elevated levels 142%, 120.5% at 7 and 14 days to 50.58% and 34.12% after 28 and 56 days respectively. The response of marine mussels to toxic stress was therefore characterised by a dramatic increase in the frequency of closures followed by shortening of the active periods.

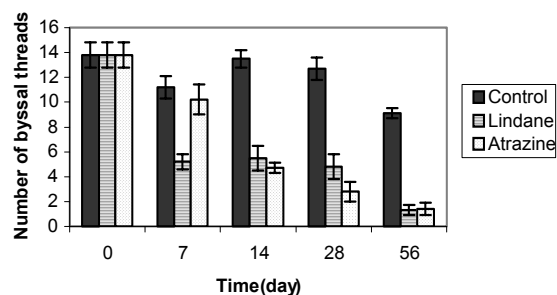
Table 3: Effect of sub-lethal concentration of atrazine on the gape (width "mm" of the shell opening measured as the distance between the rims of the valves) in the mussel, *Mytilus edulis*. The min. and max. are for the minimum and maximum gape of the mussels (n = 7). ^a significantly different from the respective control (Student's t-test, p < 0.001).

Exposure time (days)	Control of gape (min.)	Range (mm) (max.)	Atrazine of gape (min.)	Range (mm) (max.)	Control average gape (mm)	Atrazine average gape (mm)
0	25	43			34.0	
7	24	35	33.3	51	29.5	42.15
14	31	48	40.0	54	39.5	47.0
28	15	27.5	6.5	15	21.3 ^a	10.75
56	15	36	5.4	12	25.5	8.7

Effect of pesticides on byssus formation in *Mytilus edulis*: To study the chronic toxicity of atrazine and lindane, byssal threads number was measured at a range of exposure time to sub-lethal concentrations. The data for the effect of lindane on byssal thread production is summarised in figure 5. The mean number of byssal threads produced was decreased markedly and progressively after 7 days of lindane treatment compared with the control. A similar study of the effect of long term exposure of *M. edulis* to a sub-lethal concentration of atrazine on byssal threads production per mussel also showed a marked decline in byssus production with increasing time of exposure to the toxicant (Fig. 5).

The data show that the number of byssal threads produced had decreased to 50% after 14 days of atrazine treatment, while lindane has a higher effect, causing 46% reduction in byssal thread formation after 7 days of exposure. In contrast, there was a negligible change in byssus production after 7 days of exposure to atrazine. However, after 2 months of treatment, atrazine and lindane had elicited similar effects on byssus formation. The compounds tested indicate that for equitoxic concentrations, the insecticide acts more rapidly than the herbicide.

Figure 5: Effect of sublethal concentrations of lindane and atrazine on the average number of byssal threads formed in *Mytilus edulis*.



DISCUSSION

It has been suggested that behavioural responses such as bivalve shell gaping and valve movement, and physiological processes such as growth of byssal attachment might be used to provide simple assay systems for monitoring changes in environmental quality (Kramer & Botterweg 1991). Valve closure is a typical example of an escape response. Under normal conditions, mussels keep their shells open for respiration and feeding, but they keep them closed for an extended period under natural or anthropogenic stress (Kramer & Botterweg 1991). This response has been used to study a number of natural and anthropogenic effects,

including exposure to toxicants, such as trace metals (Manley & Davenport 1979), pesticides (Salánki & Varanka 1978) and other trace organics (Slooff *et al.* 1983).

Characteristic patterns can be recognised when recording the activity of *Mytilus edulis* in control (Fig 1a) and polluted conditions (Figs 1b and 1c) from the rhythmic pumping movement of shells occurring as a result of fast contractions and relaxations of the adductors during the period when shells are generally in an open state. This is the active period of the mussel, when uptake of food and oxygen occurs. From time to time, this active state is interrupted by rest periods when, as a result of tonic contraction of the adductors, the shells are tightly shut, and food and oxygen uptake are blocked. Exposure of the mussels to contamination can dramatically alter the amount of time spent in an open state, and the frequency of switching between the open (active) state and the closed (inactive) state (Kramer & Botterweg 1991). Such behaviour pattern of filter feeding molluscs increases their ability to resist, in the short term. Among the physiological mechanisms providing high resistance of molluscs to unfavourable environmental conditions is the ability of the molluscs to fall into anabiosis, when the rate of metabolism in the cells declines sharply and, consequently, oxygen uptake from the environment also decreases. Under these conditions, bivalves close their valves tightly, stop their filtering activity and decrease oxygen consumption (Karnaukhov 1979).

The continuous recording of shell activity has proved to be a good method for monitoring the effects on mussel activity associated with the bioaccumulation of pesticides from low concentrations in the surrounding water. It is the filter feeding behaviour which results in the sampling of large volumes of water which leads to the high accumulation of various substances present at low concentrations in the water. The filtering activity of bivalve molluscs such as *M. edulis* is of fundamental importance to the animals since it provides both the food and oxygen necessary for maintenance of life processes such as growth and reproduction. Any factor that decreases the time spent actively filtering will reduce the scope for growth of the animal and if sustained at a high level for sufficient time, will affect the viability of the animal. The reduced filtering activity will be evident in many of the animal's activities, ranging from oxygen consumption to growth of the byssal threads. Measurements of activity in *M. edulis* should therefore provide an early warning to environmental chemicals (El-Shenawy 1999). Salánki (1979) reported that of the various ways in which lindane may act, probably none of them influences the physiological properties and functioning of the adductors directly. This means that all the described effects are secondary lesions mediated by the nervous system in which the primary lesion occurs. The ganglia which contain collections of nerve cell bodies and are involved in regulating a wide range of activities may be affected directly or indirectly by lindane. The activity and regulatory function of the ganglia can be modified by afferent pathways arriving from peripheral and visceral receptors or by metabolites produced in the different organs. Serotonergic and catecholaminergic mechanisms play a major role in the regulation of the periodic activity of mussels centrally and at the neuromuscular level. It is highly probable that all the factors changing the behaviour of mussels act through metabolic pathways or by releasing monoamines in the ganglia and in the adductors at nerve terminals (Hiripi & Salánki 1973). Catecholamines and serotonin strongly influence the duration of the active periods for *Anodonta cygnea* L., and any compound which influences the synthesis and breakdown of monoamines would change the periodicity of activity and rest (Hiripi 1973). Heavy metals (Hg, Cs and Cu) which also inhibit the activity of mussels evoke their effect by blocking enzymes important in cell respiration, and lindane could also disturb the respiratory system in a similar manner (Salánki & Varanka 1978). Jenner *et al.* (1992) reported that the ratio of open to closed time of valve movement of *Dreissena polymorpha* exposed to atrazine was decreased. In the present investigation, the mussels reacted by decreasing the time that the valves were open with

increasing the time of exposure to lindane (Table 2). In the early stages of exposure, the mussels reacted by greatly increasing the number of valve movements (Fig. 1b).

The observed ratios of the closing/ opening time of valve movement for control mussels were in range of 0.04-0.06 throughout the whole duration of the experiments, as in *Drissena polymorpha* (Kramer et al. 1989). In the present investigation when *M. edulis* was exposed to lindane over 56 days, the ratio increased to 1.31 compared with 0.05 in control animals. Over a similar period of exposure to atrazine, the closing to opening time ratio was increased to 0.3 compared with 0.08 in the control animals.

The shell margins were eventually 5.4-12 mm apart from each other in the mussels exposed to atrazine compared with 15-36 mm for control mussels (Table 5). Shell opening in control animals was about 4 times more than in treated mussels. These observations agree with Rajagopal et al. (1997) who showed an increasing shell valve closure of *Mytilopsis leucophaeta* with increasing chlorine concentration, and the shell opening of the control mussels was about 10 times more than those treated with 1mg l⁻¹ of chlorine. The byssogenic process in bivalves is complex, involving secretion of collagen, enzyme, phenol and a sclerotised sheath from three sets of glands located in the foot (Morse & Zardus 1997). The byssal complex is a system of pedal glands, a ventral ciliated groove and muscles of the foot, that work together to form proteinaceous threads for attachment to a hard substratum. This is a complex system and coordination of the activities of the various components is essential. Furthermore, a number of environmental factors is known to affect byssogenesis in *M. edulis* including water movement, salinity, dissolved oxygen concentration, chlorination and temperature (Van Winkol 1970). Byssal thread number increases when a number of mussels is placed together compared to isolated individuals. Byssus strength increases in response to water movement and more threads are produced in water with higher flow rates (Glaus 1968). Reish & Ayers (1968) measured the number of byssus threads produced by *M. edulis* in 14 days in sea water of differing salinities and dissolved oxygen concentrations. There was no thread production at salinities below 8‰. The shells, however, remained closed at lowered chloride and dissolved oxygen concentrations. In chlorinated sea water, pedal activity is depressed and this is the principal factor affecting the rate of byssus formation. Roberts (1975) reported that byssus formation may be reduced by decreasing the pedal activity, by shell closure or by direct interference with the synthesis of combination of byssus components. Most toxins are likely to cause a reduction in byssogenesis, if only by initiating shell closure, but the apparently normal behaviour of mussels exposed to some toxins suggests a different method of interference. It can be seen that such a complex system modulated by environmental variables could act as a sensitive indicator of poisoning; for instance, any disruption of general metabolic activity or of the function of the nervous system would have a deleterious effect on normal byssus production.

Although there are different methods for assessing byssogenic activity, all techniques generally involved the rate of thread production. In the present study, the effects of lindane and atrazine on byssus formation in *M. edulis* was studied in terms of the rate of thread production and in the terms of the percentage of experimental animals attached. Both lindane and atrazine decreased byssus formation and the ability of animals to attach to each other in time-dose related manner. The mechanism(s) by which this was achieved are not clear but both pesticides reduced pedal activity by increasing the shell closure and might reduce byssus production as outlined above. Since lindane is a nerve poison, a contributory factor may interfere with muscle movements and secretions of the exocrine glands which produce the byssus. Although different toxicants may have different modes of action, the byssogenesis test offers a rapid and convenient technique for monitoring exposure to sublethal concentrations of a wide range of potentially toxic environmental chemicals, ranging from heavy metals to pesticides (Roberts 1975).

The effects of γ -HCH and atrazine on valve movement in the present study demonstrated the efficiency of the method for testing the toxicity of anthropogenic pollutants in the laboratory. The attenuation of rest periods, and/or the shortening of active periods indicate harmful effects on the behaviour of the animal. This behavioural response can be used as an indicator of the toxic contamination of water.

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