Differential Uptake of Endosulfan by the Tissues of *Mytilus edulis*

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Although increasing attention is being paid to monitoring persistent pesticides in the marine environment (Butler, 1973), information on the distribution of halogenated hydrocarbons in the tissues of marine species is scant (Nimmo, Wilson & Blackman, 1970; Nimmo, Blackman, Wilson & Forester, 1971). Nimmo et al. (1970; 1971) demonstrated differences in DDT and Polychlorinated Biphenyl (PCB) residue levels in different tissues of Penaeus duorarum. However, Butler (1971) failed to demonstrate consistent differences in pesticide residues in two body components of Pecten irradians when gills were analysed separately from the remaining tissue.

The effects of sub-lethal concentrations of Endosulfan on spawning and condition of the common mussel, Mytilus edulis, have been reported in an earlier paper (Roberts, 1972). The patterns of uptake and elution of the pesticide during, and after exposure to a range of concentrations of Endosulfan, indicated slower equilibration with the pesticide and more rapid elution of residues at higher concentrations. In addition, high residue levels were recorded in the digestive gland of the scallop, Chlamys operculatis, after exposure to Endosulfan. In view of these facts it was suggested that the rapid assimilation and elution of large quantities of Endosulfan was due to ingestion and egestion of the pesticide adsorbed on particulate matter. Therefore this investigation was undertaken to compare the rates of assimilation and elution of Endosulfan by different tissues of M. edulis, and results presented here demonstrate differences between the tissues investigated, consistent with the earlier suggestion.

Materials and Methods

80 mussels, approximately 60 mm in length, were exposed to 0.1 mg Endosulfan l^{-1} for 36 days, in duplicate 40 l tanks through which the test solution was siphoned at about 20 l a day. They were then transferred to clean seawater for a further period of 23 days. A weekly sample of 3 mussels was taken from each tank, and the tissues divided into the following components for residue analysis: the digestive gland; the mantle plus gonad; the gills;

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and the remaining tissue (consisting of pedal retractor muscles, foot, and anterior and posterior adductor muscles). Tissue samples from each tank were pooled, to provide sufficient material for analysis, homogenized, and 5 g wet weight of each tissue was taken for residue estimation.

The pesticide was extracted in petroleum ether using the technique developed by de Faubert Maunder et al. (1964) and cleaned on a Florisil chromatographic column (Burke & Mills, 1963) to remove co-extracted compounds which might interfere with subsequent estimation. Analysis was carried out using an Aerograph Hy-Fi chromatograph, model 600D (Wilkins Instrument & Research Inc.) fitted with an electron capture detector. The column used was all glass, with a glass lined injector, and consisted of 5% Dow 11 on 60-80 Chromosorb W at an oven temperature of 185°C and nitrogen carrier gas flow rate of 38 ml min^{-1}. Residue levels were taken as the mean of two injections, corrections being made from the ratio of the solvent peaks. Endosulfan exists as 2 sterioisomers of which the α-form accounts for 2/3 and the β-form 1/3 of the active principle in commercial preparations. Concentrations were calculated from summated peak areas of both isomers and recovery was estimated to be 74% efficient.

Results

Fig. 1 shows the distribution and concentrations of Endosulfan residues in tissues of C. operculatis and M. edulis after 2 weeks exposure to 0.1 mg Endosulfan l^{-1}. The residue values given represent the total isomer content; pesticide levels were not divided into isomeric components because of problems associated with recovery of β- Endosulfan (Roberts, 1972). This isomer was not detected, however, until after 36 days exposure suggesting differential uptake of the isomers.

The major site of concentration of Endosulfan is the digestive gland. Both C. operculatis and M. edulis assimilated a similar amount in this organ, 6.1 & 6.2 $\mu g\ g^{-1}$ respectively, and this was considerably in excess of that in any other tissue. Residue concentrations in the foot, anterior and posterior adductor muscles of M. edulis were similar to those in the adductor muscle of C. operculatis (0.75 & 0.88 $\mu g\ g^{-1}$ respectively). There were, however, marked differences between the residue levels in the other tissues of the 2 species. The level in the gills of M. edulis (2.1 $\mu g\ g^{-1}$) was almost 5 times that in the gills of C. operculatis (0.46 $\mu g\ g^{-1}$) while in the gonad and mantle the position was reversed and residue levels in these tissues of C. operculatis (3.8 & 1.34 $\mu g\ g^{-1}$) exceeded that for the equivalent combined tissues of M. edulis (0.66 $\mu g\ g^{-1}$). Mean tissue residue levels for C. operculatis and M. edulis, estimated from the summated values for the separate tissues, were 2.57 and 2.85 $\mu g\ g^{-1}$ wet weight respectively and therefore compared favourably in spite of the differences in distribution.

Fig. 2 shows the changes in residue levels in the tissues of mussels during 36 days exposure to 0.1 mg Endosulfan l^{-1} and on subsequent removal to clean seawater for 23 days. Mean tissue residue levels, estimated from the summated values for separate tissues, are similar to total tissue residue levels determined for

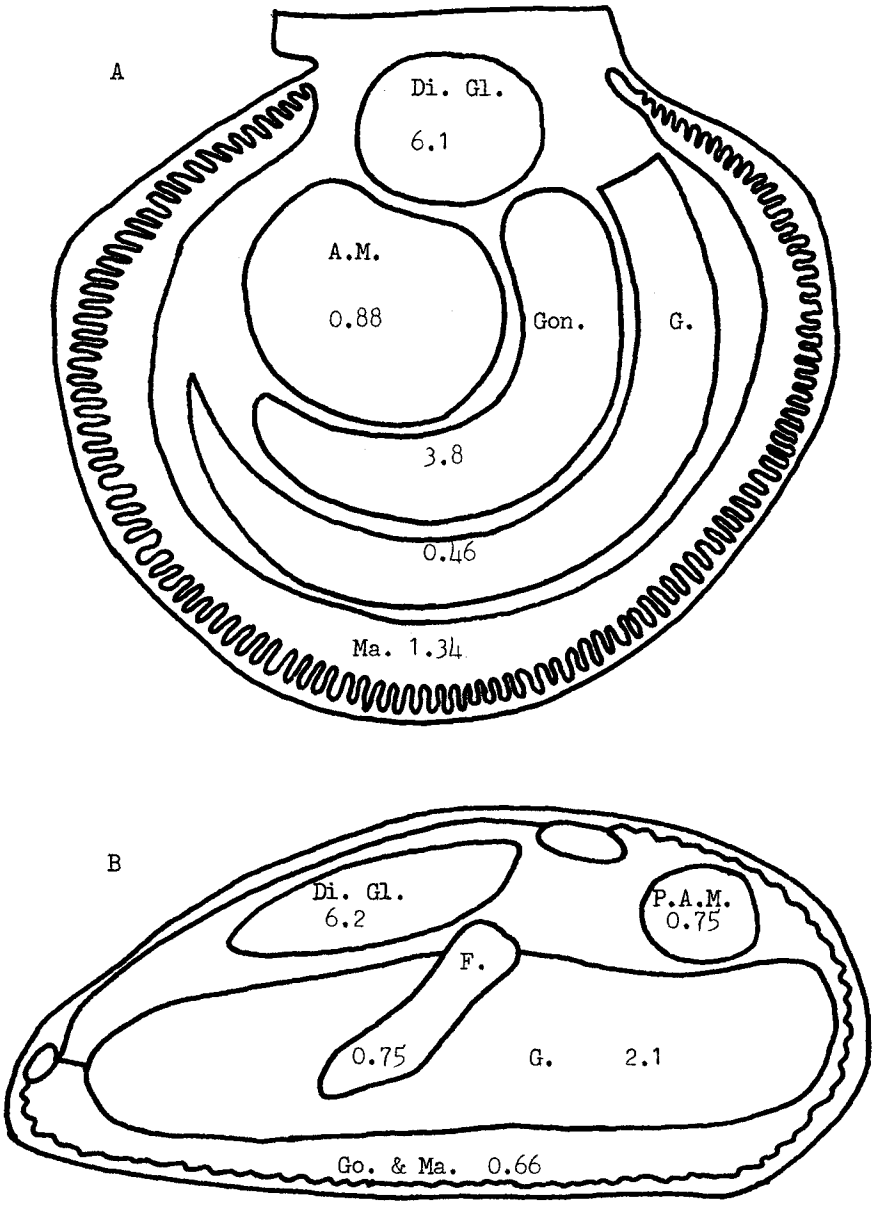

Fig. 1. The distribution of Endosulfan in A. Chlamys opercularis and B. Mytilus edulis, after 2 weeks exposure to 0.1 mg Endosulfan l^{-1}. Results, expressed in $\mu g\ g^{-1}$ wet weight, represent total isomeric composition. Tissues analysed: Adductor Muscle (A.M.); Digestive Gland (Di. Gl.); Foot (F.); Gill (G.); Gonad (Gon.); Mantle (Ma.); & Posterior Adductor Muscle (P.A.M.)

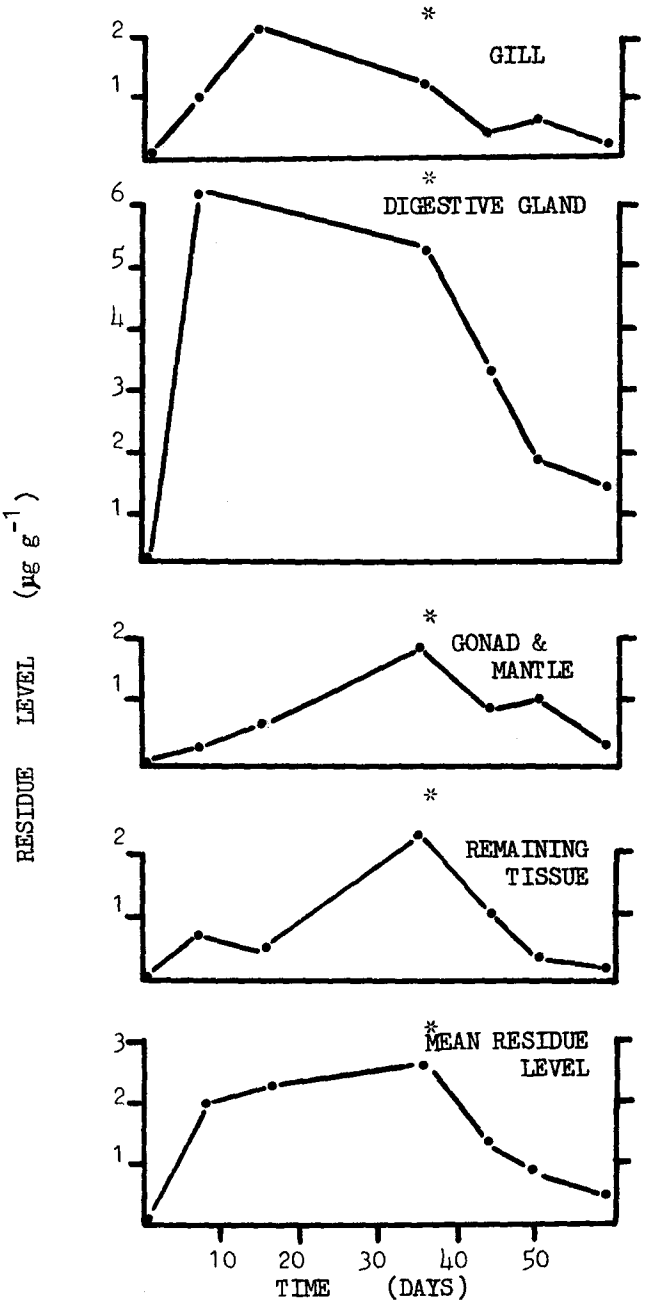

Fig. 2. The assimilation of Endosulfan by different tissues of Mytilus edulis exposed to 0.1 mg l^{-1} of the pesticide in sea-water. Results (µg g^{-1} wet weight) represent total isomeric composition. * - Point at which exposure to Endosulfan was terminated.

mussels exposed to 0.1 mg l^{-1} in the similar investigation described previuosly (Roberts, 1972).

The most obvious feature of Fig. 2 is the rapid and large accumulation of pesticide by the digestive gland. The maximum residue level in this organ was reached after 7 days exposure, whereas in all other tissues this was not reached until significantly later. In the gills the maximum was recorded after 15 days exposure, while in the gonad plus mantle and the remaining tissue, the highest level was found in the last sample during exposure, indicating that it continued to rise throughout this period.

On removal to clean seawater residue levels declined fairly rapidly initially in all tissues but showed the greatest decline in the digestive gland during the first 14 days of elution. During the final 6 days of elution the rate of residue loss was similar for all tissues.

Discussion and Conclusions

Assimilation of Endosulfan could result from direct absorption across exposed tissue surfaces or by ingestion of the pesticide adsorbed on particulate matter. In bivalves the most likely path of entry of adsorbed pesticides is via the gills, since these are the original site of particle accumulation; this would be followed by assimilation in the digestive gland. The gills of M. edulis, however, did not assimilate Endosulfan to the levels recorded for the digestive gland and the rise to the maximum level was slower. These differences possibly reflect an initial concentration of pesticide contaminated particles on the gills with further concentration in the digestive gland and subsequent assimilation of the pesticide into the gill tissue.

The initial rapid loss of residue from the digestive gland probably reflects the loss of particulate matter with adsorbed pesticide, although this organ retains the highest residue level of all tissues, even after 23 days of elution. This suggests that the digestive gland is the major site of Endosulfan assimilation and merits more detailed investigation, possibly using radiotracer techniques.

Assimilation of pesticide into the gonad, mantle, and remaining tissues could result either from direct absorption or by assimilation via the body fluids. The slow rate of assimilation by these tissues and the apparent importance of the digestive gland in pesticide uptake by the whole animal suggests the latter as the more likely route, although it is probable that there is some assimilation by direct absorption. The fairly rapid fall in residue levels in these tissues on transfer to clean seawater suggests direct diffusion from exposed surfaces as the more likely path of loss.

Differences exist in the rate of assimilation of pesticides by different species of mollusc (Butler, 1971) and although no great differences existed between the total mean residue levels in M. edulis and C. opercularis after similar periods of exposure to 0.1 mg Endosulfan l^{-1} there were noticeable differences in the distribution of the pesticide. Butler (1971) suggested the amount

of exposed body surface as a possible cause of different rates of uptake by different bivalves and this could perhaps account for the differences in the residue distribution recorded between the two species. One other factor which, in combination with anatomical differences, could certainly cause differences in total uptake, if not in distribution, is the difference in filtration rates between species. Walne (1972) demonstrated significant differences between specific filtration rates for different species of similar dry weight. The differences demonstrated for rates of uptake of pesticides by M. edulis and Mercenaria mercenaria (Butler, 1971) however, are not consistent with this view, since both have similar specific filtration rates (Walne, 1972).

The general picture obtained from studies on mammals and birds is that the highest concentrations of DDT occur in adipose tissue. This is also true for fish (Holden, 1962). The distribution of Aroclor 1254 (PCB) in the shrimp P. duorarum is also related to the amount of lipid in the tissues (Nimmo et al. 1971). In the bivalve Tivela stultorum the digestive gland has the highest lipid level (Giese, 1969), and if this also the case in M. edulis and C. opercularis the high residue levels in the digestive glands of these species may be an indication that, in contrast to warm blooded animals (Deema, Thompson & Ware, 1966; Maier-Bode, 1968), Endosulfan is stored in the fat.

Investigators studying the uptake of chlorinated hydrocarbons by aquatic organisms have proposed several mechanisms of uptake. Holden (1962), concluded that in the brown trout, Salmo trutta, the greatest intake of DDT, in the absence of DDT-contaminated food, was through the gills. Nimmo et al. (1971), pointed out that both water and food are sources of Aroclor 1254 to P. duorarum and although they did not conclude which contributed more to uptake they suggested that Aroclor adsorbed on detritus was probably ingested by the shrimp. Wildish and Zitko (1971), however, propose direct absorption across branchial and general body surfaces as the most important method of uptake of Aroclor 1254 by Gammarus oceanicus, and regarded the part played by ingestion of adsorbed PCB as minimal. The present study, however, points to the importance of ingestion in the uptake of pesticides. In view of their low solubilities (Muirhead-Thompson, 1971) and the fact that they are readily adsorbed on organic detritus and suspended silt (Butler, 1971) it seems likely that ingestion of pesticide adsorbed on suspended matter is the major route of entry of pesticides into particulate feeding invertebrates, and that direct absorption plays only a secondary role.

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