LABORATORY AND FIELD INVESTIGATIONS ON THE EFFECTS OF TRIBUTYLTIN ON THE OYSTER, *OSTREA EDULIS*

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Tributyltin (TBT) is the main active ingredient in organotin-based antifouling paints. It is possibly the most toxic substance that is intentionally introduced into the marine environment and its ecological impact at extremely low levels, particularly on molluscs, has been confirmed through a number of laboratory and field investigations (AXIAK *et al.*, in press). The present work reports on cytopathological effects in the adult oysters *Ostrea edulis* on laboratory exposure to environmentally realistic levels of TBT, with special reference to digestive cell atrophy. Moreover, the likely ecological significance of this effect is discussed and evaluated through field studies. These investigations form part of a programme of evaluation of biological impact of TBT in the Mediterranean.

Adult specimens of *Ostrea edulis* were exposed to nominal concentrations of TBT chloride (dissolved in ethanol) of 100 and 10 ng l^{-1} in unfiltered sea water. Exposure was conducted for 96 hours with test mixtures being kept aerated throughout the whole period and renewed every 24 hours. Oysters were left unfed during the experiment, which was conducted at 17°C with a photoperiod of 12:12 dark to light hours. After exposure, surviving animals were fixed in Bouin's reagent, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax. 7um thick sections were then stained with Erlich Haematoxylin and Eosin. The effect of TBT on the digestive cells (Type I) of the diverticula of the digestive gland was assessed by measuring the heights of such cells under a magnification of X 1000.

The mean height of digestive cells decreased from 13.44 um (sd: 2.25) in controls to 11.43 um (sd: 2.41) in animals exposed to 10 ng l^{-1} of TBT, with effects being found to be statistically significant at $P < 0.001$ with Tukey's multiple comparison tests at this and all other levels of exposure.

In another experiment, shell thickening was evaluated using a shell thickness index (STI) in *Ostrea* collected from five coastal sites around the major harbour area (Grand Harbour and Marsamxett) in Malta (Central Mediterranean). Malta's major yacht marinas and ship-repairing yards are located there. Oysters were also collected from a clean reference site which is only exposed to limited boating activities. These sites differed markedly in the levels of organotins in sediments as well as in the water column as measured by GC-FPD (AXIAK *et al.*, in press). Mean seasonal levels of TBT in superficial sediments at the different sites, expressed as ng Sn per g dry weight ranged from 18 to 210 for Grand Harbour; from 18 to 410 for Marsamxett; and below detection limit at a reference site outside the harbours. Levels of TBT at 1m depth in the water column ranged from 8 to 120 for Grand Harbour; and from 8 to 40 ng Sn /g DW for Marsamxett.

STI which is the ratio between valve length to valve thickness, has been used as an index of abnormal shell growth in bivalves. Low STI values are indicative of shell abnormalities which are usually due to the formation of various types of minute chambers within the shell matrix (BRYAN and GIBBS, 1991). The mean STI value for the lower valve of animals collected from the reference site was found to be 21.65, while mean STI values (for the lower valve) for animals collected from Grand Harbour and Marsamxett ranged from 9.9 to 10.5 and from 8.5 to 11.8, respectively. STI for both upper and lower valves indicated that shell abnormalities were significantly higher in areas within the Grand Harbour and Marsamxett Harbour than in the reference site.

Digestive cells of bivalves are known to undergo atrophy on exposure to a wide range of contaminants. This cell atrophy is normally correlated with catabolic metabolism and reduced bioenergetic balances of bivalves. However such cytological stress in *Ostrea* as reported here, has never been recorded for any contaminant at such low environmental concentrations. It is here proposed that reduced bioenergetic balance and the consequent reduction in body weight may lead to a shell chambering effect in this test species as shown in the field experiment. Shell abnormalities in *Ostrea*, as indicated by STI were partly due to the formation of minute water-filled chambers within the shell. New shell deposition by an animal with reduced somatic growth may lead to the formation of such chambers. Such shell abnormalities may be part of a general stress syndrome which may be elicited by a range of contaminants and not just TBT. Nonetheless, the laboratory experiments reported here, provide evidence that this species is particularly sensitive to low levels of TBT (10 ng/l) in sea water, exhibiting significant atrophy in digestive cells.

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