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COMMENT

Reply to comment 'Tributyltin and imposex: no uncertainty shown' by Ruiz et al.

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We welcome the comments provided by Ruiz et al. (1998 in this issue) and the opportunity to improve and clarify our paper (Davies et al. 1997). In the following reply we respond to their main points.

(1) 'Davies et al. state that "the degree of imposex [in *Nucella lapillus* (L.)] did not exceed levels which caused sterility in any treatment group" (in abstract p 191 and again on p 203 but worded differently).'

Table 5 is correct. In the text, 'sterility' is used in relation to the reproductive capacity of the sample group. The important point is that at the time eggs were produced (about 9 mo into the experiment) the levels of imposex were not high enough to halt egg laying (animals in all treatment tanks reproduced) and that at that time a negligible proportion of the females had attained VDSI stages 5 or 6, as defined by Gibbs et al. (1987).

(2) '[T]he discussion of the results of this study and previous investigations showed considerable uncertainty about the association of lower degrees of imposex and TBT contamination (p 203).'

There is no question about the ability of TBT to cause imposex in gastropods. However, Oehlmann et al. (1996) stated that 'The formation of a penis or other male sex characteristics in females was already known before the first considerable use of TBT-based antifouling paints in the early 1960s'. As discussed by Matthiessen & Gibbs (1998), 'any substance able to cause elevations in testosterone titres ... has the potential to produce this type of sexual disturbance'. It is therefore conceivable that very low levels of imposex may occur as a result of the effects of factors other than TBT. We do not disagree that the dominant cause of imposex in marine gastropods is exposure to TBT compounds.

We agree that there has been no independent confirmation of the experimental data in Nias et al. (1993). The high control mortalities, apparent strong imposex response (penis growth) to copper, but lack of convincing dose-response relationship raises many unanswered questions. The authors suggest that other factors, such as acetic acid, or the experimental system (an environmental stressor) may have been important.

We explored the possibility of cross-contamination between dosed and controlled tanks. Our observations related to the covariance of seasonal patterns in the rather low levels of imposex observed in samples collected simultaneously from the same beach in Loch Ewe as the experimental animals, and animals in control tanks. Seasonal changes in penis length in dogwhelks have previously been described by Stroben et al. (1996). Such changes may reflect seasonal hormonal cycles, or be related to other factors such as food availability or temperature. We described the relationship between temperature and feeding rate (p 201).

The covariance between the control tanks and the field populations, and the comparable scale of changes seen in field populations at various other locations in Loch Ewe in 1987–1990 (Bailey 1991) led us to conclude (p 198) that no cross-contamination could be identified in the experimental system. A cause of the low levels of imposex in the beach samples was not established. Possibilities must include very low concentrations of TBT from remote sources, together with other factors as discussed above. A detailed discussion of the possibilities of the presence of, or potential for, a naturally produced pseudohermaphroditism would be too detailed for this comment. However, Matthiessen & Gibbs (1998) provide a detailed review of relevant studies of the endocrine processes leading to imposex and related phenomena. Factors which affect the levels of testosterone may result in the subsequent induction of the growth of a penis in female snails and be important at low levels of imposex.

There is a general absence of experimental evidence at very low tributyltin levels. Early reports of penis-like

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structures around the mid-1960s and early 1970s may be useful to provide indications of the effect of low levels of TBT on the development of imposex. Some reports raised the possibility of other confounding factors, for example Smith (1971) reported 'a bump over the right tentacle in the normal position for a penis' in the American mud snail and observed that 'Parasitism of snails by larval trematodes seems to suppress imposex expression'. We agree with Ruiz et al. that there is a need for good experimental work at TBT concentrations below 2 ng l^{-1}. The relationship between tributyltin and imposex at low levels of imposex is not clear.

Our phrase '...considerable uncertainty about the association...' does not state that tributyltin at low concentrations does not cause imposex, it merely questions whether the relationship is as straightforward at low degrees of imposex as has been found for higher concentrations of tributyltin and imposex (Gibbs et al. 1987). We state (p 203) that 'The results of our study support the earlier data indicating that low concentrations of below 2 ng l^{-1} promote the development of imposex in *Nucella lapillus*.'

(3) Ruiz et al. commented on the points: 'an experiment which exposed dogwhelks collected from Loch Ewe, an "uncontaminated population" (p 203), to "clean sea water (TBT < 1.5 ng l^{-1})" (i.e. their detection limit, p 192). They concluded that "[for imposex] NOEC [no-observed-effect concentration] is less than 2 ng TBT l^{-1}. Reliable experimentation at concentrations below this is presently difficult, and clear establishment of a NOEC would require significant improvements in chemical analysis and tank maintenance techniques" '

Ruiz et al. agreed with the NOEC for imposex being below 2 ng TBT l^{-1} and suggested that even if a technique with a lower detection limit had been used (such as was published in 1997 by Yamada et al.) the NOEC may still be given as 'below' a certain limit. We do not dispute this, but with a lack of experimental evidence at concentrations less than 2 ng TBT l^{-1}, no other statement about the NOEC can be made. The UK Environmental Quality Standard (EQS) for tributyltin in seawater is 2 ng TBT l^{-1} (p 191; Water Research Center 1988). Therefore, to say that the NOEC was less than this value, implies that above 2 ng TBT l^{-1} seawater effects would be observed. It does not relay any comment about what may occur below 2 ng TBT l^{-1} seawater. In saying '[the] clear establishment of a NOEC would require significant improvements.. ' we suggest that the NOEC would lie well below the 2 ng TBT l^{-1} EQS.

Reliable experimentation below 2 ng TBT l^{-1} is difficult, as evidenced by the scarcity of experimental studies. There the operational and analytical difficulties (both chemical and biological) to overcome. Gibbs et al. (1987) showed good relationships of the RPSI [mean bulk of the female penis (length^3) expressed as a percentage of the mean bulk of the male penis (length^3)] and the VDSI stages in animals from field sites with the tributyltin concentrations in the ambient seawater of the sampling sites and the body tissues of the animals. Indeed the imposex level shown in the animals, especially adults a few years old, will reflect historical exposure to tributyltin as well as the present situation. We are not aware of any experimental study which has produced clear dose-response relationships at low levels of imposex and TBT exposure. Our experiment was designed to investigate the sub-lethal responses of the animals in the dosed tanks. It was not designed to investigate the onset of imposex at less than 2 ng TBT l^{-1}. A completely different experimental design would be required for this purpose.

(4) Ruiz et al. asked 'How can Davies et al. be certain that their control water did not contain sufficient TBT to elicit the imposex response?'

The imposex levels in the 2 control tanks and the quarterly beach samples were similar throughout the experiment, indicating that, whatever influence the actual TBT concentration in the control water had on the development of imposex in the animals in the control tanks, the seawater in Loch Ewe had a similar effect on the field samples from the beach. It should be noted that at all sampling times the RPSI and VDSI in the animals from the tank with the lowest dosage of TBT, that is 2 ng l^{-1}, were greater than those in animals from the beach or either of the control tanks (Table 5). Any contribution from the control water to the imposex response in the dosed tanks would be minor in comparison to the dosage of TBT the animals in tanks 3 to 6 received.

(5) Ruiz et al. commented 'They describe a population as "uncontaminated" when, in fact, they mean one that should be strictly considered as a population contaminated at a level below their detection limit of "0.02 mg kg^{-1} .. wet tissue weight".'

The term 'uncontaminated' was used once, on p 203, to mean unexposed to the contaminant tributyltin under the experimental system.

(6) 'It has to be noted that the improvements in chemical analysis which Davies et al. plead for have indeed been achieved in the last decade, and routine protocols for tissues currently yield TBT detection limits below 10 ng Sn g^{-1} dry weight (e.g Ide et al. 1997, Ruiz et al. 1998). In addition, it has long been recognised that pollution reports should control and assure the quality of data by means of the use of certified reference materials (material for TBT has been available from the Japanese NIES since 1990).'

The detection limits that we quoted were conservative (1.5 ng TBT l^{-1} seawater, and 0.02 mg TBT kg^{-1}

wet weight or 20 ng TBT g^{-1} wet weight) and were achieved in routine analysis. We are therefore confident of the data at these levels. These were adequate for our purposes and were typical of good methods available when the work was carried out.

We hope that this reply clarifies the comments made by Ruiz et al. While we welcome this correspondence, we hope that it does not detract from the main theme of the paper, the sublethal effects of tributyltin at concentrations in seawater of 2 to 128 ng l^{-1}.

LITERATURE CITED

Bailey SK (1991) Tributyltin contamination in Scottish coastal waters. PhD thesis, Napier University, Edinburgh

Davies IM, Harding MJC, Bailey SK, Shanks AM, Länge R (1997) Sublethal effects of tributyltin oxide on the dog-whelk *Nucella lapillus* (L.). Mar Ecol Prog Ser 158:191–204

Gibbs PE, Bryan GW, Pascoe PL, Burt GR (1987) The use of the dog-whelk, *Nucella Lapillus*, as an indicator of tributyltin (TBT) contamination. J Mar Biol Assoc UK 67: 507–523

Ide I, Witten EP, Fischer J, Kalbfus W, Zellner A, Stroben E, Waterman B (1997) Accumulation of organotin compounds in the common whelk *Buccinum undatum* and the red whelk *Neptunea antiqua* in association with imposex. Mar Ecol Prog Ser 152:197–203

Matthiessen P, Gibbs PE (1998) Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. Environ Toxicol Chem 17(1):37–43

Nias DJ, McKillup SC, Edyvane KS (1993) Imposex in *Lepsiella vinosa* from southern Australia. Mar Pollut Bull 26(7): 380–384

Oehlmann J, Stroben E, Fioroni P (1996) New facts about tributyltin-induced imposex in prosobranchs: general aspects. Malacol Rev Mollusc Reprod (Suppl) 6:149–256

Ruiz JM, Quintela M, Barreiro R (1998) Tributyltin and imposex: no uncertainty shown. Mar Ecol Prog Ser 170: 293–294

Smith BS (1971) Sexuality in the American mud snail *Nassarius obsoletus* Say. Proc Malac Soc Lond 39:377–378

Stroben E, Oehlmann J, Schulte-Oehlmann U, Fioroni P (1996) Seasonal variations in the genital ducts of normal and imposex-affected prosobranchs and its influence on biomonitoring indices. Malacol Rev Mollusc Reprod (Suppl) 6:173–184

Water Research Center (1988) Proposed environmental quality standards for List II substances: organotins. Water Research Center, Madmenham, WRC Tech Rep TR 255

Yamada H, Takayanagi K, Tateishi M, Tagata H, Ikeda K (1997) Organotin compounds and PCBs of livers in squid collected from coastal waters and open oceans. Environ Pollut 96:217–226