

## COMMENT

**Tributyltin and imposex: no uncertainty shown****J. M. Ruiz\*, M. Quintela, R. Barreiro**

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The deleterious effects of tributyltin (TBT) from antifouling paints on the marine environment is still a matter of lively scientific research and political debate in the late 1990s, with TBT midway between worldwide regulation and possible total phase-out. For instance, this journal has recently published 3 works dealing with TBT accumulation and the masculinization of female neogastropods ('imposex'), both in the field (Ide et al. 1997, Ruiz et al. 1998) and under experimental conditions (Davies et al. 1997). We comment here on 2 important questions raised by this last paper.

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). This conclusion does not accord with the data given in Table 5 (p 196), where it can be noted that a considerable proportion of the females in tank 5 (32 ng TBT l<sup>-1</sup>) and in tank 6 (128 ng TBT l<sup>-1</sup>) reached VDSI (Vas Deference Sequence Index) stage 5 (i.e. 32.7 and 24.5%, respectively). Since it is said on p 193 that the VDSI classification devised by Gibbs et al. (1987) was followed, and this sequence clearly defines VDSI 5 females as sterile, there must be an error. The implications of such a sterilisation of nearly one third of the adult females treated are considerable since old mature females are known to be far less responsive than young maturing stages (Gibbs et al. 1988). It may be that Table 5 contains misprints; alternatively, a serious misinterpretation has been made. Given the wide dissemination of the abstracts of papers, we feel a public correction is called for.

Our main concern revolves around the statement that 'the 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). This is literally correct: it is indeed the discussion which casts doubts, not the results themselves. Faced with the fact that 'a low degree of imposex ... was found in the pre-study female snails' (p 202), Davies et al. can only think of 2 possibilities: (1) 'This may either reflect a natural, background level of this phenomenon ... which is unrelated to pollution ... and possibly related to the exposed aspect of the sampling site (Oehlmann et al. 1991)' or, reworded, 'the snails exhibit a "natural" level of pseudohermaphroditism (Bryan et al. 1986)'; and (2) 'or indicate that the snails have previously been exposed to some substance that has caused the onset of imposex development' and, continuing along this line of thought, 'Other factors, however, such as copper, paint matrix and environmental stress, have been found to induce imposex in the gastropod *Lepsiella vinosa* (Nias et al. 1993)'. Firstly, it has to be said that the use Davies et al. make of the literature is misleading; as far as we are aware none of the authors cited, nor any of their co-workers, has suggested the possibility of a natural level of imposex in *Nucella lapillus* populations: (1) Oehlmann et al. (1991) just contended that the high variability of imposex expression in specimens from Brittany (not the occurrence of imposex itself) is related to the nature of the coast; and (2) Nias et al. (1993), after a work which did not include any chemical analyses of water or tissues, merely 'suggested ... other factors [those above] ... may also induce this deformity' (abstract op. cit.), they did not prove it; moreover, they state that 'there may have been accidental cross-contamination of the water, equipment or mussels. This possibility cannot be excluded since extremely low concentrations of TBT have been linked with the development of imposex' (Nias et al. 1993, p 384). And this wise acknowledgement is the third hypothesis we think Davies et al. (1997) should have considered because it is the most

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likely explanation for their observations and the key issue of the whole argument.

In addition to the bibliographic issue, we question Davies et al.'s interpretation of the results of an experiment which exposed dogwhelks collected from Loch Ewe, an 'uncontaminated population' (p 203), to 'clean sea water (TBT < 1.5 ng l<sup>-1</sup>)' (i.e. their detection limit, p 192). They concluded that '[for imposex] NOEC [no-observed-effect concentration] is less than 2 ng TBT l<sup>-1</sup>. 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'. Whilst we agree with the NOEC lying below 2 ng TBT l<sup>-1</sup>, we would add that nobody knows for sure how far below. Even if a technique rendering a detection limit of 0.2 ng l<sup>-1</sup>, such as that used by Yamada et al. (1997), had been applied, the results would be inconclusive because the NOEC may well lie in the pg l<sup>-1</sup> range. How can Davies et al. be certain that their control water did not contain sufficient TBT to elicit the imposex response? This argument can also be applied to the tissue residue topic. 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<sup>-1</sup> wet tissue weight (p 193) [or, assuming a dry:wet ratio of 3 and a TBT:Sn ratio of 2.5, they cannot detect TBT residues below 24 ng Sn g<sup>-1</sup> dry weight]. 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<sup>-1</sup> 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 National Institute for Environmental Studies since 1990].

In summary, Davies et al. have not refuted the considerable body of evidence demonstrating that TBT is the only agent known to induce imposex in *Nucella lapillus* populations. Future studies of this ubiquitous phenomenon will require modern methods and a rigorous interpretation of data for any significant advances to be made.

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