TOXICITY CHARACTERISATION OF MARINE EFFLUENT DISCHARGES AND SURFACE WATERS

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Successful assessment of the risk posed by hazardous substances in the marine environment requires techniques to identify the compounds responsible. Described is the characterisation of toxicants present in a marine effluent discharge and in industrially impacted surface waters. Both characterisation processes engaged toxicity identification evaluation (TIE) procedures that have been adapted for marine samples using marine bioassays.

1. Marine effluent discharges

A full USEPA type TIE was performed on a sample obtained from a pharmaceutical plant discharge into the marine environment. Initial toxicity was assessed by testing with the *Tisbe battagliai* and *Skeletonema costatum* bioassays (Figure 1&2). Since the sample showed significantly greater toxicity to *S. costatum*, this bioassay was used for the remainder of the investigation.

Figure 1. Tisbe battagliai

Figure 2. Skeletonema costatum

Phase 1 manipulations showed the toxicity to be removed by both C18 solid phase extraction (SPE) and activated carbon with little effect from any other manipulations (Figure 3). Crude fractionation using a C18 SPE column eluted with different concentrations of methanol in water produced one toxic fraction (Figure 4) which when fractionated further by HPLC produced a single toxic fraction corresponding to a non-polar organic compound(s) (Figure 5).

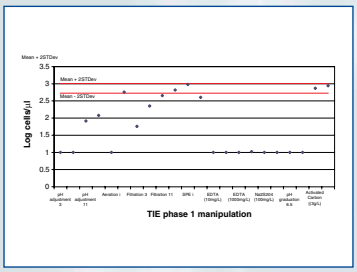

Figure 3. Phase 1 effluent TIE manipulations and results

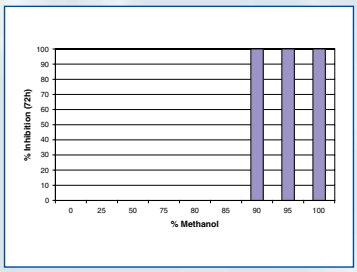

Figure 4. Phase 2 coarse fractionation (toxins eluted in the 90-100% methanol fractions)

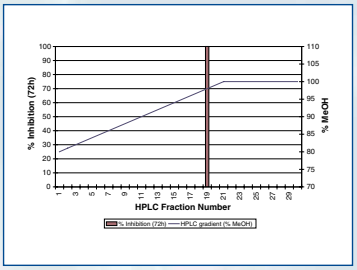

Figure 5. Finer fractionation by HPLC. Elution of toxic fraction is shown.

Analysis of this fraction by GC-MS, however, failed to identify any candidate toxicant. This may be due to the toxic agent being unsuitable for analysis by GC (i.e. not sufficiently volatile). This data is sufficient for the needs for Toxicity Reduction, however, further work is required before the toxicant can be positively identified.

2. Industrially impacted UK estuaries

A generic TIE procedure specifically developed to identify toxic organic compounds was applied to water samples collected from industrially impacted UK estuaries. As a first step in attributing causality, the organic load of bulk water was isolated using a solid phase extraction system. Where acute toxicity to *T. battagliai* was demonstrated in the sample extract, the complex mixture of organic contaminants in the sample were fractionated by HPLC, tested using *T. battagliai*, and the compounds responsible for toxicity identified by GC-MS (Figure 6).

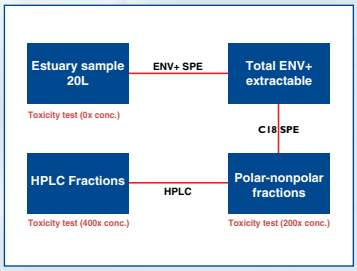

Figure 6. Extraction and fractionation of organics in water

The system was evaluated with a range of reference compounds of widely differing polarity (Figure 7). The method allowed trace contaminants to be concentrated up to 400 times environmental concentrations before candidate toxicants were measured (Table 1).

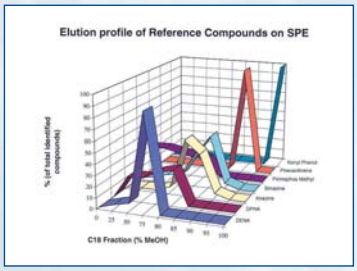

Figure 7. Elution profile of reference compounds on SPE

Compound	48h LC_{50} (µg/L)	Uses
Nonylphenol	100 (*D. magna*)	Surfactant metabolite
2,4-Dimethyl phenol	2100 (*D. magna*)	Agricultural chemical and pesticide
4-Chloro-3, 5-xylenol	200 (*T. battaglia*)	Pesticide metabolite/antiseptic
Pentachlorophenol	100 (*T. battaglia*)	Wood preservative
Tetrachlorophenol	290-500 (*D. magna*)	Pesticide and industrial chemical intermediate
Trichlorophenol	1000 (*D. magna*)	Pesticide and industrial chemical intermediate
Dichlorophenyl isocyanate	unknown	Chemical intermediate
Difluron*	20 (*D. magna*)	Insecticide
2, 3, dichloronaphthalenedione*	25 (*D. magna*)	Agricultural chemical and pesticide
Methyl(5-hydroxy (1H) benzimidazol-2-yl) carbamate*	unknown	Pesticide metabolite

* Tentative identifications

Table 1. List of compounds identified as present in the toxic fractions isolated by HPLC. A high concentration to toxicity ratio implicates the compounds listed as the cause of toxicity

This approach allows compounds with a demonstrated hazard in the marine environment to be identified. A range of compounds are present in UK estuaries at concentrations sufficient to be the cause of sub-lethal toxic effect, and are not included in current routine monitoring programmes. The source of this toxicity is specific to individual estuaries. It is suspected that chlorinated phenols are a major contributor to unexplained toxicity on the lower reaches of the River Tyne, alkylphenol surfactant metabolites on the River Tees and dieldrin on the River Mersey. Other compounds are also contributing to the unexplained toxicity but remain unidentified. The identified compounds could now be added to existing and future monitoring programmes.

Not only does this approach provide a valuable investigative tool to attribute the cause of harmful effects in both effluents and estuaries, with a fit for purpose approach, it can also prove a cost effective solution to environmental problems.

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