

Since its development as a tool for the characterisation of effluent discharges by the USEPA, toxicity identification evaluation (TIE) has been applied to a variety of different scenarios. Here we present three examples of how TIE has been applied at CEFAS to assess freshwater environmental problems.

### 1. Identification of toxicants in STW effluent

Effluents discharged from sewage treatment works (STWs) contain a complex cocktail of chemicals derived from both industrial and domestic activities. At present monitoring is limited to a narrow range of chemical and physical properties. Direct toxicity assessment (DTA) seeks to change this by regulating discharges by their toxic effect. An integral part of DTA is the application of TIE to characterise toxic effluents in an attempt to identify the toxic agent(s) present.

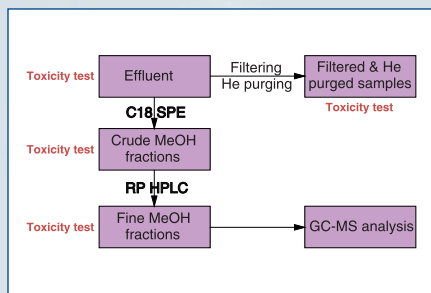


Figure 1. TIE scheme for isolating organic compounds in STW effluent

The organic toxicants present in STW effluents from industrial areas in the North of England were characterised using a TIE approach. The organic compounds were isolated and fractionated using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) (Figure 1). All fractions were tested using *Daphnia magna* (Figure 2). GC-MS analysis of each individual toxic fraction identified chlorfenvinphos, diazinon and nonylphenol as being present in sufficient quantities to be the cause of toxic effect.



Figure 2. *Daphnia magna*

The source of chorfenvinphos and nonylphenol was tracked to the textile industry since chlorfenvinphos is a common sheep dipping treatment and nonylphenol a metabolite of non-ionic surfactants that are used during fleece washing.

### 2. Toxicity characterisation of river water following a major fish kill

Following a major fish kill on the Kennet and Avon Canal, Berkshire, UK, in 1998 an indicative accelerated TIE investigation was performed on canal water in an attempt to rapidly characterise the toxic agents present.



Figure 3. *Oncorhynchus mykiss* (fingering rainbow trout)

Water samples were screened for toxicity using fingering rainbow trout (~ 3 cm, *Oncorhynchus mykiss*, Figure 3) and shown to be toxic at 3-10% after 4h. Several candidate groups of compounds were eliminated following Phase 1 TIE manipulations (Figure 4). Further novel manipulations in Phase 2 showed that the toxic agent was labile.

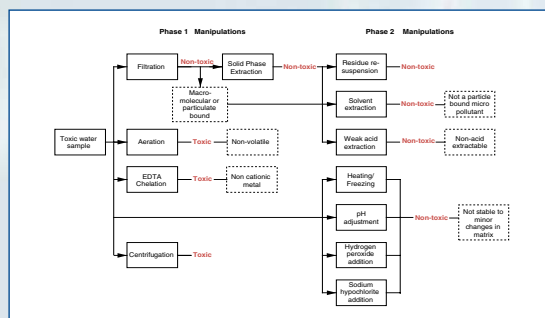


Figure 4. TIE manipulations performed on toxic water samples from the Kennet and Avon canal

By using this simple set of procedures it was possible to deduce the following:

- The active component was not a metal
- The active component was not a volatile substance
- GFC filtration caused a reduction in toxicity, however, some long-term toxicity was still evident
- Centrifugation did not remove toxicity
- Re-suspension of the filtered particles caused only 20% mortality after >60h
- The active component was not stable to extraction in dichloromethane, methanol or weak acid (10% acetic acid)
- The active component was not stable to heat (> 40°C)
- The active component was not stable to freezing
- The active component was not stable after adjustment of the sample pH to 4
- The active component was not stable to the addition of hydrogen peroxide
- The active component was not stable to the addition of sodium hypochlorite

These TIE procedures were performed over a very short time scale and at the fraction of the cost of specialist chemical analyses. The general perception of TIE procedures is that they are very time consuming and expensive. Although the TIE approach is largely aimed at anthropogenic contaminants, even when confronted with biological material, cost-effective toxicant characterisation is possible. An advantage of the approach is that it allows a more effective use of time and resources in that a narrower range of specific and specialist analyses are required in order to identify the cause of toxic effect. Although this short TIE study did not identify the exact compounds concerned, remediation options were successfully identified.

### 3. Toxicity characterisation of storm waters

The transient movement of pesticides at biologically active concentrations during storm events is considered to be a cause of biological impoverishment in headwater streams. The programme of work described, seeks to identify compounds that are the cause of toxic effect during such events. The approach adopted has been to employ toxicity identification evaluation (TIE) procedures to identify compounds with a demonstrated toxic effect using procedures specifically directed towards concentrating, isolating and attributing toxicity to organic classes of contaminants in storm waters. Samples were collected from an English headwater stream during a storm event and the organic load isolated by means of solid phase extraction (SPE). Bioassay of the SPE extract at x100 whole water concentrations, confirmed that the samples contained substances toxic to *Daphnia magna* (Table 1). Simplification of the SPE isolate by reverse phase HPLC produced thirty fine fractions which were also tested for toxicity. The cause of toxicity in Sample A was isolated to one fraction (#18) whilst Sample B showed toxicity in fractions #17 and #26-28 (Figure 5). Extraction of the fine fractions demonstrating toxicity using dichloromethane, followed by GC-MS analysis identified nonylphenol, endosulfan sulphate and pendimethalin as present in the second sample with the majority of the explained toxicity attributed to nonylphenol (NP). It is suspected that the source of NP is the use of nonylphenolpolyethoxylate as a carrier in agricultural pesticide formulations.

Table 1. Toxicity of storm water samples (concentrated x100) to *D. magna*

Sample	<i>Daphnia magna</i> (Immobilisation) (95% CL)			
	EC <sub>50</sub> 24h	EC <sub>50</sub> 48h	TU <sup>†</sup> 24h	TU <sup>†</sup> 48h
A	>56%	47 (43-51)%	>1.8	2.1 (2.0-2.3)
B	48 (42-54)%	24 (22-27)%	2.0 (1.9-2.4)	4.2 (3.7-4.5)

<sup>†</sup> Toxicity unit (TU) = 100%/EC<sub>50</sub>

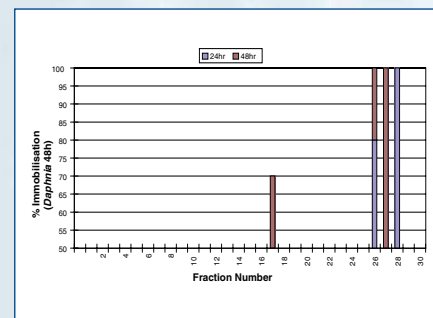


Figure 5. *D. magna* toxicity profile of a stormwater sample following fine fractionation by HPLC

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