

Introduction

As part of an ongoing review of the Water Framework Directive (WFD), the European Commission (EC) is considering "effects based tools" (EBTs) for use as an alternative to, or in combination with, the monitoring of individual substance concentrations. One potential approach for incorporating EBTs into environmental monitoring programmes is to apply them on extracts from passive sampling devices. Toxicity evaluation of time-integrated passive sampler extracts offers a pragmatic solution for applying EBTs to surface water monitoring and for estimating the effects of chemical mixtures. Here, we present an initial assessment of the EBTs that could be applied with passive sampler extracts for monitoring surface waters associated with refinery discharges.

Aims

- Identify a range of EBTs that can be applied for surface water quality monitoring (marine and freshwater), combining whole organism responses assessing adverse effects at the population level with cell based assays indicating exposure to specific substances or groups of substances;
- Evaluate the assays with respect to their validation maturity, pedigree for use in the assessment of environmental samples, applicability for use with passive sampler extracts, and response to hydrocarbons;
- Suggest a suitable suite of reliable and relevant assays for use in the monitoring of potential effects of hydrocarbons in the receiving environment.



Methods

- A list of EBTs was compiled based on recent reviews^{1,2,3,4,5};
- Assays were screened based on their commercial availability, validation maturity, application to environmental samples and suitability for use with passive sampler extracts (i.e. low sample volumes);
- Twenty-two EBTs were shortlisted for a more detailed literature review as shown in Figure 1;
- Scientific studies based on the actual use of the assays for environmental monitoring were used to evaluate performance, interpretation in relation to hazard and mode of action, and their response to hydrocarbons;
- The literature review for whole organism responses focused on more novel assays but standard, well established methods (e.g. ISO methods) were also considered as part of the final evaluation;
- Where assays were similar those with greater regulatory applicability were given priority.

Results

EBTs that could be used to assess the effects of passive sampler extracts from surface waters associated with refinery discharges are given in Table 1.

Table 1: EBTs that could be applied with passive sampler extracts for monitoring surface waters associated with refinery discharges.

Assay	Assay type
Toxicity to <i>Allivibrio fischeri</i> (ISO 11348)	<i>In vivo</i>
Multi-species microbial toxicity test	<i>In vivo</i> (freshwater/marine)
Miniaturised <i>Daphnia acute</i> test (OECD 202)	<i>In vivo</i> (freshwater)
Microplate Algal growth tests (OECD 201 or ISO 10253)	<i>In vivo</i>
Bivalve embryo development test (ICES No.54)	<i>In vivo</i> (marine)
Cytotoxicity in a Rainbow trout cell line	<i>In vivo</i> / adverse effect
Miniaturised AMES test	Genotoxicity
umuC	Genotoxicity
ER activation assay	Endocrine Disruption
AR activation assay	Endocrine Disruption
AhR activation assay	Metabolism
AhR activation assay (with more specificity for PAHs)	Metabolism
AREc32 activation assay	Oxidative stress

Discussion

Whole organism assays – The assays cover representative bacteria, algae, invertebrates and fish. The inclusion of a multi-species microbial toxicity test may assist in improving taxonomic coverage. The fish cell line cytotoxicity assay is suggested in preference to a Fish Embryo Test (FET) test based on ethical considerations and its practicality (e.g. sample volume requirements).

Endocrine disruption – For the oestrogen receptor (ER) and androgen receptor (AR), human cell based assays were generally more sensitive than yeast based assays. An assay for thyroid activity was not included in the final list because it was not considered sufficiently well developed/ validated.

Mutagenicity – Five assays were considered covering both direct and indirect genotoxic effects. A miniaturised AMES test is recommended because it provides a direct measure of the occurrence of fixed mutations and is more specific for genotoxicity (less false positives) compared with alternative assays based on DNA repair. This could however be used in conjunction with the umuC assay, which is more readily quantifiable and sensitive, to provide information on the genotoxic mechanism of action.

Oxidative stress – The AREc32 was the most mature assay in terms of validation and use with environmental samples.

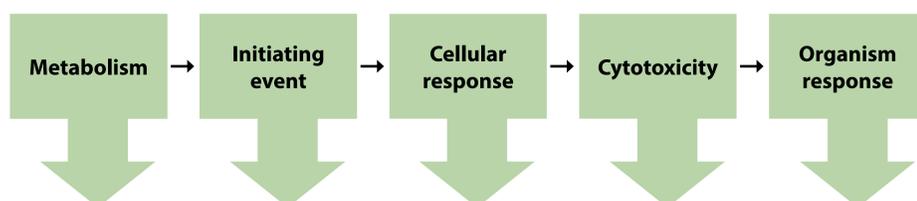
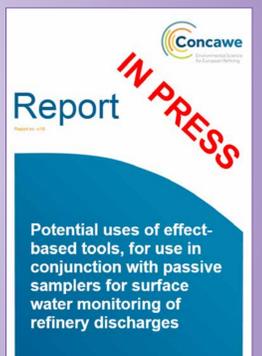
Metabolism – The shortlisted metabolism assays were all indicative of aryl hydrocarbon receptor (AhR) mediated induction of cytochrome P4501A (CYP 1A) because of the specific response of some PAHs. Two types of AhR activation assays were included to differentiate between the AhR effects of different PAH fractions.

Conclusions

The highlighted assays provide a robust starting point for the development of a suite of EBTs for the specific application of monitoring surface waters associated with refinery discharges. However, the list is not intended to be definitive, and a flexible approach is recommended whereby EBTs are trailed monitoring programmes, results evaluated and their suitability reassessed, on an ongoing basis.

Bioassays which might provide new insights on exposure or a mechanism of toxicity are continually being developed, and therefore it is suggested that such tests be considered for inclusion within the battery of tests, either to investigate specific endpoints and substances or because, following validation, they prove superior to those currently discussed.

For detailed information on the assessment of the assays and approach applied to select them, please see the full Concawe Report: "Potential uses of effect-based tools, for use in conjunction with passive samplers for surface water monitoring of refinery discharges" (in press; soon to be available on the Concawe website).



Assay Type	Metabolism	Initiating event	Cellular response	Cytotoxicity	Organism response
In vitro assay	AhR activation (with and without more specificity for PAHs); EROD activity in fish cell line	ER activation; YES assay; AR activation (two assays); YAS assay; TTR assay; Zebrafish Toxarray	AMES fluctuation; umuC ¹ ; p53-pathway activation ¹ ; Activation of hGADD45a ¹ ; ARE c32 activation ² ; Nrf2 pathway activation ² ; Micronucleus	Cytotoxicity in RT Gill W1	Zebrafish FET; <i>D. magna</i> metabolic activity; <i>C. elegans</i> (48h); Multi-species microbial toxicity
In vivo assay					

Figure 1: Initial shortlist of bioassays chosen for a more detailed literature review.

References

- Hamers et al. (2016). Final report of the LRI-ECO23 project November 2016.
 Brack et al. (2016). Science of the Total Environment 544:1073–1118
 Common Implementation Strategy (CIS) (2014). Technical Report – 2014 – 077
 Di Paolo et al. (2016). Water Research 104:473-484
 Schriks et al. (2015). Report from "Demonstration of promising technologies to address emerging pollutants in water and waste water (DEMAU)" WP41 October 2015.

Abbreviations

- AR – Androgen receptor
 ARE – Antioxidant Response Element
 AhR – Aryl hydrocarbon receptor
 ER – Oestrogen Receptor
 EROD – Ethoxyresorufin-O-deethylase
 FET – Fish Embryo Toxicity
 hGADD45a – human GADD45a
 PAH – Polycyclic Aromatic Hydrocarbons
 RT Gill W1 – Rainbow Trout Gill cells
 TTR – Transthyretin
 YAS – Yeast Androgen Screen
 YES – Yeast Oestrogen Screen