

BRANCHED ALKYL BENZENES: A MAJOR TOXIC COMPONENT OF UNRESOLVED COMPLEX MIXTURES BIOACCUMULATED IN MUSSELS?

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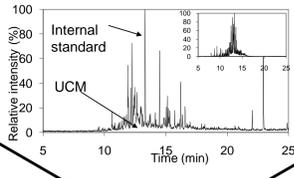
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Introduction

Recent research using comprehensive two-dimensional gas chromatography – time-of-flight mass-spectrometry (GCxGC-ToF-MS) identified branched alkylbenzenes (BABs) as a major component of some unresolved complex mixtures of hydrocarbons bioaccumulated in the tissues of N. Sea mussels, *Mytilus edulis*, (Booth *et al.*, 2007) that were previously found to have poor health status (Widdows *et al.*, 1995). Acute aqueous toxicity tests (72h semi-static exposures) using a commercially available complex mixture of C₁₂₋₁₄ BABs containing over 340 compounds (Fig. 1) revealed that they possessed a similar toxicity, in terms of mussel clearance rates (a major component of the 'Scope for Growth' health index), to that of the linear C₈ alkylbenzene, 1-phenyloctane, and established an aqueous BAB EC₂₀ of 7 µg L⁻¹ and a tissue BAB EC₂₀ of 10.5 µg g⁻¹ dry weight. Acute exposure may not necessarily be relevant to the exposure conditions that mussels and other organisms experience, therefore further research was required to assess the effect of longer term exposure at low aqueous conditions, and to assess the ability of mussels to recover from exposure to BABs.



Methods

- 14-day exposure to 5 µg L⁻¹ of C₁₂₋₁₄ BABs
- Triplicate exposure
- Daily water exchanges
- Continuous food supply (alga: *Isochrysis*)
- Clearance rates measured
- Rates statistically compared to solvent (0.005% acetone) and seawater control mussels, using analysis of variance (ANOVA)
- Cellular viability (neutral red retention) assessed
- Subsample of mussels placed in clean seawater for 5 days to assess their ability to depurate and recover
- Mussel tissue extracted by alkaline saponification
- Tissue extracts were quantified and profiles of bioaccumulated compounds compared using GC-MS and GCxGC-ToF-MS
- Tissue extracts from 3-day exposed mussels (Booth *et al.*, 2007) re-analysed by GCxGC-ToF-MS and compared with results from 14-day exposure

Results

- Mussels exposed to BABs compounds for 14-days at 5 µg L⁻¹ accumulated concentrations of BABs similar to mussels exposed for 3-days at 40 µg L⁻¹
- Tissue burdens calculated by GCxGC-ToF-MS (31 µg g⁻¹ dry wt.) were similar to GC-MS (46 - 47 µg g⁻¹ dry wt.)
- Clearance rates significantly ($P \leq 0.05$) reduced (Fig. 2)
- Cellular viability not significantly affected ($P > 0.05$)
- Incomplete recovery following 5 days in clean seawater
- Tissue concentration of BABs within depurated mussels reduced by ~50%
- Results consistent with narcosis mode of action.

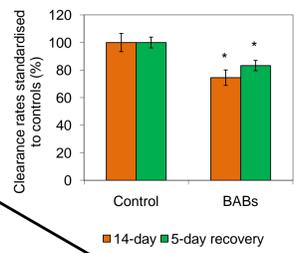
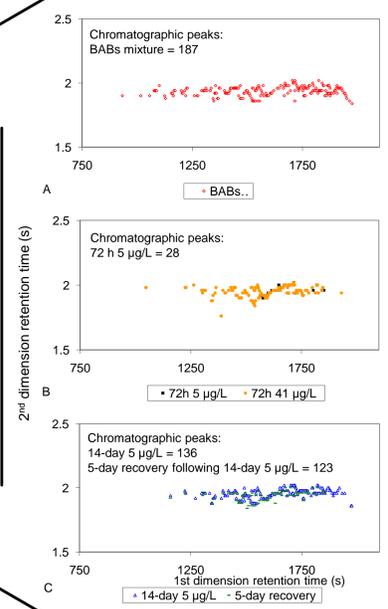


Fig. 2 Effect of 14 days exposure and 5 days depuration to BABs mixture * = significance $P \leq 0.05$

Results



Profile of accumulated compounds similar to BABs mixture standard, especially when mussels chronically exposed (Fig. 3)

Fig. 3. Chromatographic peak marker plots derived by GCxGC-ToF-MS) analysis of a pure complex mixture of C₁₂₋₁₄ branched alkylbenzenes (BABs, A) and those accumulated in mussel tissues following 72 h exposure (B), and 14 days exposure plus 5 day depuration (C)

Conclusions

- Broad range of alkylbenzene isomers accumulated by mussels
- Accumulated compounds not fully depurated
- Hence, potential for BABs to accumulate in wild populations resulting in reduction in health status
- Results consistent with UCM profile of wild mussel populations with poor health status

References

- The research presented herein has recently been submitted to *Environ. Toxicol. & Chem.*
- Scarlett, A., Rowland, S.J., Galloway, T.S., Lewis, A.C. and Booth, A.M., Resolution of unresolved complex mixture hydrocarbon components by GCxGC-ToF-MS reveals chronic sublethal effects associated with branched alkylbenzenes bioaccumulated by mussels. Submitted to *Environ. Toxicol. & Chem.*
 - Booth, A.M., Sutton, P.A., Lewis, C.A., Lewis, A.C., Scarlett, A., Chau, W., Widdows, J., Rowland, S.J., (2007) Unresolved Complex Mixtures of Aromatic Hydrocarbons: Thousands of Overlooked Persistent, Bioaccumulative, and Toxic Contaminants in Mussels. *Environ. Sci. Technol.*, 41(2), 457-464.
 - Widdows, J., Donkin, P., Brinsley, M.D., Evans, S.V., Salkeld, P.N., Franklin, A., Law, R.J., Waldock, M.J., (1995) Scope for Growth and Contaminant Levels in North-Sea Mussels *Mytilus-Edulis*. *Marine Ecology-Progress Series*, 127(1-3), 131-148.