C17 Use of Cytochrome P-450 1A Response to Extracts from Semi-Permeable Membrane Devices to Identify Sources of Organic Pollutants

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After attending this presentation, attendees will understand a new and powerful method of linking field evidence of biological impacts to the causative contaminant source in studies conducted in support of environmental litigation.

This presentation will impact the forensic community and/or humanity by demonstrating increasing the appreciation of the power derived from combining molecular biological biomarker metrics (e.g. cytochrome P450 1A induction) with methods for passively concentrating environmental contaminants to narrow the field of plausible suspect sources in environmental forensic cases.

Assessing complex mixture toxicity by summing their concentrations may not be sufficiently realistic to evaluate or predict the consequences of exposure. An effective alternative involves the modification of a well-documented form of mimetic chemistry, the semi- permeable membrane device (SPMD), and the exposure of test animals to its contents. This method allows the evaluation of the effects of those bioavailable compounds present, including those whose analysis is difficult with their breakdown products and metabolites, as encountered in situ. This approach facilitates an evaluation of toxicity for each sample and site as a unit. In situations where chronic adverse effects from organic contaminants on aquatic biota are evident but the source is not obvious, this approach may be used to help isolate the proximate cause. This approach was applied to the Prince William Sound, Alaska, the site of the 1989 Exxon Valdez oil spill, to evaluate which of several prospective pollution sources best account for evidence of chronically depressed populations of sea otters and some sea ducks there.

SPMDs for the standard 28-day deployment period were deployed, recovered, and concentrated accumulated organic contaminants, and injected aliquots of extracts containing the contaminants in juvenile rainbow trout (*Oncorhynchus mykiss*). The juvenile trout were sacrificed after 2 or 7 days, the livers excised and examined with the ethoxyresorufin-*o*-deethylase (EROD) bioassay. The results demonstrate that even after fifteen years, there is enough bioavailable oil in formerly oiled intertidal habitats of Prince William Sound to elicit a marked induction of CYP1A. The induction potential from oiled sites are comparable to those from a boat harbor (hot control), and are significantly elevated above environmental controls from sites that were not oiled. These results indicate that oil bioavailability is real, and can be evaluated with this technique.

Measurements of CYP1A induction were used to compare the potency of lingering oil from the 1989 Exxon Valdez oil spill (EVOS) with pollutants from alternative sources. Arrays of SPMD were deployed at intertidal sites where EVOS oil remains, at other intertidal sites impacted by present or historical human activity, at salmon streams to assess pollutants imported to PWS by migrating salmon, at Constantine Harbor where a suite of natural petrogenic hydrocarbons is present in intertidal sediments, and at randomly-selected sites to assess inputs from atmospheric transport or from ambient seawater. CYP1A induction was measured by the EROD assay applied to homogenized rainbow trout livers two days following injection. SPMD extracts were also analyzed for polycyclic aromatic hydrocarbons (PAH) and for a suite of persistent organic pollutants (POP) including chlorinated pesticides and PCBs. The magnitude of CYP1A induction caused by SPMD extracts from the EVOS sites ranged from 28 – 72 pmol/mg/min, much greater than elsewhere (1.5 – 6.5 pmol/mg/min; median 2.5). The CYP1A induction from the oiled sites was significantly ($P < 0.01$) related to total PAH concentrations of the extracts, and these all fingerprinted to EVO.

Of the nine human activity sites (hatcheries, old mine sites), only one current use site registered significant loads of PAH and stimulated a CYP1A response. At 45 un-impacted sites (salmon streams, non-oiled areas, random marine sites), background concentrations of PAH and POP stimulated a weak (< 6.5 pmol/mg/min) to negligible CYP1A response. These results indicate that POPs are negligible as CYP1A induction agents in PWS, as are PAH associated with historical human use sites (except at Sawmill Bay), whereas oil from the EVOS remains a potent CYP1A induction agent.

The EROD assay of exposed trout livers proved to be a very sensitive response to the accumulated contaminants. Absent readily identifiable local pollution sources, most of Prince William Sound was so clean that trace levels of EROD-inducing agents initially present in the SPMDs as received from the manufacturer were significantly lowered through losses to the environment at the end of the 28-day exposure period. Hence, in Prince William Sound, this method provided a very sensitive method for evaluating relative contributions from a manifold of pollution sources to the adverse biological effects observed in sea otters and ducks.

Exxon Valdez Oil Spill, Semi-Permeable Membrane Device, Cytochrome P450 1A

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