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The Use of Semipermeable Membrane Devices (SPMDs) to Concentrate Inducers of Fish Hepatic Mixed Function Oxygenase (MFO)

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Abstract

Semipermeable membrane devices (SPMDs) are sampling and concentrating devices comprised of a thin polyethylene membrane containing a small quantity of triolein. They have previously been used to sample air, water and sediments and have concentrated fish tainting compounds from pulp mill effluents. The ability to induce mixed function oxygenases (MFOs) is a property of a variety of organic effluents, but the compound(s) responsible for induction have not been identified. We wanted to see if SPMDs would accumulate the MFO-inducing chemical(s) from pulp mill effluents and oil refinery effluents. Dialysates of effluent-exposed SPMDs induced ethoxyresorufin-O-deethylase (EROD) activity in a fish (*Poeciliopsis lucida*) hepatoma cell line, PLHC-1. In pulp mill effluents and oil sands mining and refining wastewaters, potencies varied greatly, from a few to thousands of pg TCDD-EQ/g SPMD. Low levels of inducers were seen in four pulp mills on the Athabasca R., and higher levels at one New Brunswick bleached sulphite and two Ontario bleached kraft pulp mills. The highest levels of MFO inducers were in SPMDs deployed for 14 days in wastewater from an oil sands upgrading facility, as well as SPMDs deployed at two sites on Athabasca River tributaries in the oil sands area. This suggests that natural erosion and weathering, as well as industrial processing of the oil sands, can release potent MFO inducers. Background (reference) induction by SPMD extracts ranged from non-detectable (<1) to 20 pg TCDD-EQ/g SPMD. Reactive clean-up of one of the bleached kraft mill effluent-exposed SPMD extracts on a sulfuric acid/silica gel column resulted in loss of the inducer(s), which suggested a polyaromatic hydrocarbon-type of inducing chemical(s), rather than a dioxin or furan inducer. SPMD deployments proved useful in the detection of inducers within the pulp mill process streams as extracts of SPMDs exposed to untreated bleached sulphite effluent were ten to twenty times as potent as those from secondary-treated effluent. Little is known about the nature and identity of the MFO inducers from pulp mill and refinery effluents, but the use of SPMDs as concentrators of MFO-inducing substances appears a promising avenue for future research.

Introduction

Mixed function oxygenases (MFOs) are detoxification enzymes that increase in content and activity after exposure to certain compounds. The increase in MFO activity usually indicates an increase in the amount of enzyme in cells and is referred to as induction (Okey, 1990). Increased hepatic MFO activity is frequently observed in fish sampled from waters contaminated by pulp mill effluents (Rogers *et al.*, 1989, Munkittrick *et al.*, 1991, Hodson *et al.*, 1992) or oil drilling and refining (Payne *et al.*, 1987; Sherry *et al.*, 1995). The enzymes measured (usually ethoxyresorufin-O-deethylase, or EROD, and arylhydrocarbon hydroxylase, or AHH) are part of the P450IA1 family of enzymes, which can increase in concentration and activity following exposure to chemicals such as polynuclear aromatic hydrocarbons (PAHs), planar PCBs, chlorinated dibenzo-*p*-dioxins, chlorinated dibenzofurans, chlorodiphenylethers, chlorinated napthalenes and plant flavones (Safe 1990, Giesy *et al.*, 1994, Okey *et al.*, 1994). Since these compounds are highly toxic and since increased MFO activity in the livers of fish exposed to pulp mill effluents is often found along with other changes in reproduction, growth, pathology and physiology of the fish, it is important to know the nature and concentration of compounds affecting the MFO system.

Although induction of fish hepatic MFO is a common finding in fish downstream of pulp mills, low levels of dioxins and furans in both effluent and biota suggested these compounds were not responsible for the elevated MFO. Coupled with the findings of elevated MFO in fish downstream of mills that do not use chlorine, these data suggest the presence of an unknown inducer(s). The task of isolating the chemical(s) responsible for MFO induction out of the thousands of chemicals present in pulp mill effluent is formidable. Research on the nature and identity of MFO inducers from pulp mill and refinery effluents has used several approaches: 1. Chemical fractionation of effluent, 2. Extraction of tissues of exposed fish, 3. Knowledge of known inducers and testing of pure compounds, and 4. Use of semi-permeable membrane devices (SPMDs) to concentrate inducing chemicals from pulp mill effluents.

This paper will review the last approach: the use of SPMDs as concentrators of MFO inducers. We will focus on several case studies where SPMDs have been used to concentrate MFO inducers from effluents and waters, and the advantages and limitations of these devices.

Review

SPMDs

Semipermeable membrane devices (SPMDs) are flat polyethylene membrane tubes (standard size: 91 cm long x 2.5 cm wide low density polyethylene tube, wall thickness 0.80 μm) containing a thin film (1 mL, 0.915 g) of purified synthetic triolein, a substance that constitutes a major fraction of the neutral lipid of fish. The SPMD was developed by Huckins *et al.* (1990) as a passive *in-situ* sampler that concentrates neutral organic chemicals with log K_{ow} 1, size 10 Å, and a molecular weight of about 600 or less. Freely dissolved chemicals of appropriate polarity diffuse through the polyethylene membrane and into the triolein (Huckins *et al.*, 1990).

Originally, SPMDs were used as concentrating devices for subsequent chemical analyses and estimation of chemical contamination. Comparison of the chemicals present and their concentrations in SPMDs, water, sediment or biota at the deployment site, expands our knowledge of the site and the concentrating properties of SPMDs, but tells us nothing about the toxicity of that particular chemical mixture. An advantage of a direct bioassay of the SPMD extracts is that the biological potency, which takes into account potential chemical interactions, is determined.

Analyses ofdialysates of SPMDs by MFO assays was first initiated by Don Tillitt (Huckins *et al.*, 1996). Measuring MFO induction by SPMD extracts (reviewed in Hodson *et al.*, 1996) can enhance understanding of the chemical properties of the inducing substance. The maximum ethoxyresorufin-O-deethylase (EROD) activity observed in the cells gives clues about the type(s) of inducing chemicals: chlorinated dioxins and furans cause greater maximum EROD activity (100-200 pmol/mg/min) than PAH-type inducers (10-30 pmol/mg/min). Reactive clean-up or other types of treatment of the extracts can identify which classes of chemicals are or are not responsible for MFO induction. Passing SPMD extracts through a H_2SO_4/silica column destroys labile compounds such as PAHs, while dioxins and furans are not affected. Thus, both the presence and the magnitude of the cell's response can give important information about the nature of the chemicals concentrated by the SPMD.

The use of SPMDs has several benefits over traditional water sampling (of large volumes by solvent extraction) and fish caging. Among these are the ease of handling and deployment. SPMDs can be freely suspended in water or effluent, or can be submerged inside a simple holding cage or protective device. SPMDs have an advantage over caged fish in that SPMDs are able to sample "hostile" effluents and waters, where fish may not survive due to toxicity, high or low temperatures, high or low pH, low dissolved oxygen or high particle concentration. Because SPMDs are deployed for one to several weeks, they provide a time-integrated sample of the water or effluent, which is often more meaningful than a single grab sample.

An additional advantage of the SPMD is the selectivity of the membrane to sample only freely dissolved neutral organic molecules. The permeability of the membrane is similar to that of a fish gill integument in terms of size selectivity. The uptake of chemicals is restricted at higher molecular weight sizes in a fashion similar to that of fish gills (Huckins *et al.* 1996). The SPMDs sample only the freely dissolved portion of the chemical in the water. This is extremely important in that the freely dissolved chemical is that fraction of the whole water concentration that is bioavailable, not bound to dissolved or particulate organic carbon, and thus available for uptake into an organism. The fraction of chemical bound to organic carbon in the water column is not readily taken up by an organism, yet this may constitute the largest portion of the chemical when"whole water" is analyzed. Therefore, the SPMD allows estimation of either the "free", bioavailable chemical concentration(s) or the potency of those chemicals when they are tested in a biological assay, such as the PLHC-1 cells. There is continued debate surrounding the bioavailable concentration of chemicals in water under various environmental conditions and the SPMDs offer an elegant method to further understand this issue.

The fact that the SPMD is inert with regards to metabolism of chemicals can be an advantage or a disadvantage. The advantage comes from the fact that the amount of a chemical in the SPMD

is a better reflection of an aquatic organism's exposure during that time period. For example, the assessment of PAH exposure of fish is difficult to gauge accurately because the PAHs are metabolized and thus are not found as parent compounds in fish flesh. The metabolic process may render the PAH labile and available for elimination or it may enhance the toxicity of the compound. Therefore, if it were possible to measure the amount of a compound that a fish were truly exposed to during a given period, it may be possible to predict dose-response relationships under field conditions. The SPMD offers such an opportunity.

A disadvantage of the SPMD is the fact that any type of facilitated transport or uptake of chemicals from the food is not accounted for with the SPMD. If the foodchain is an important route of exposure of an organism, the SPMDs can not mimic this path of uptake.

SPMD Sampling and Exposure Methods

The units commonly used in association with the SPMDs (L/day) are based on standard size SPMD containing 1.0 mL of triolein and having a weight of approximately 4.9 g (0.9 g triolein and 4 g of polyethylene membrane) and the assumption that the uptake of that volume of water is 100 % efficient. The units may be converted to the more conventional units of uptake rate constants, L/g/day, by normalization to the weight of the SPMD used in the particular study.

Concentration of compounds into SPMDs is related to the compound's K_{ow} and molecular size. The higher the K_{ow}, the more compound will accumulate in the lipid, and the longer it will take for the concentration in the lipid to reach equilibrium with the concentration in the water. SPMD uptake rate can be expressed as the litres of water sampled by the SPMD per day. For polyaromatic hydrocarbons, sampling rates range from 0.3 L/day/SPMD (naphthalene) to about 5 to 6 L/day/SPMD (chrysene and pyrene) (Huckins *et al.*, 1996). For compounds larger than 4 or 5 aromatic rings, size becomes a limiting factor. The high K_{ow} favours accumulation of the compounds in the triolein, but the size of the molecules impedes rapid diffusion across the polyethylene membrane, and uptake is slowed. SPMDs concentrate large PAHs, such as benzo[g,h,i]perylene and benzo[a]pyrene at rates of about 2 to 4 L/day/SPMD (Huckins *et al.*, 1996).

During SPMD exposures, temperature and flow regimes often differ among sites. Differences in water velocity past the membrane should not influence the concentrations of inducers in the SPMDs. The rate-limiting step for uptake of compounds into SPMDs is diffusion across the membrane (Huckins *et al.* 1996). Water temperatures can vary between sites and effluents and for some compounds (such as pesticides) this can influence uptake into SPMDs (Huckins *et al.*, 1995a). But for rigidly-structured PAHs, the influence of temperature on SPMD uptake is minimal. Since known inducing compounds fall into the category of rigidly-structured, planar molecules the influence of temperature differences on inducer uptake by SPMDs is expected to be minimal.

Long SPMD deployments can result in significant membrane fouling. Fouling of the membrane may affect sampling rate, but effects are not as dramatic as expected after visual examination of fouled membranes. Huckins *et al.* (1996) found fouled SPMDs (left in the Upper Mississippi River for 58 days) concentrated phenanthrene at 65 % the rate of unfouled SPMDs. Fouling

slows uptake of compounds from effluents and river waters, but in deployments of up to 14 days this effect may be a slight underestimation of potency. For river and effluent deployments of SPMDs, exposure time should be long enough to collect an integrated sample, and to allow sufficient concentration of inducers, but not so long that membrane fouling interferes with the results.

Deployment of SPMDs must be done carefully and precautions must be taken to prevent contact of SPMDs with contaminated field equipment. SPMDs should be handled using latex gloves and the deployment should be performed as quickly as possible to reduce exposure to air (Petty *et al.*, 1993) and contaminants during handling. Trip blanks are SPMDs exposed to air and handled for the same amount of time as deployed SPMDs, then re-sealed in the can for transport to the laboratory.

Deployment or holding devices should protect the SPMDs while withstanding the conditions of exposure in the river or effluent, and allowing sufficient exchange of water. Deployment devices used can be as simple as plastic laundry baskets or metal barbecue rotisserie baskets. In less turbulent conditions fibreglass screen mesh tubes can be used. For fast-flowing river deployments weighted steel tubes have been successfully used. Upon removal, SPMDs are removed from deployment devices, sealed in new, solvent-rinsed paint tins and frozen until extraction and biological analyses.

Analyses of SPMD Extracts

In the lab, analysis of the SPMDs involves physical cleaning, solvent dialysis of compounds from the SPMDs and concentration of extracts for dosing to fish liver cells grown in culture (Figure 1). The SPMD surfaces are physically cleaned by scrubbing in water prior to methanol and hexane rinses. Membranes are dialysed for 48 h at 17 °C in 1 L hexane. The dialysate is rotary evaporated to about 5 mL and filtered through anhydrous sodium sulphate. The eluent is concentrated to 1 mL under nitrogen, then size exclusion HPLC is used to separate compounds of interest (chromatographic column: 250 x 22 mm of phenogel (Phenomenex, Torrance, California) adsorbent, isocratic mobile phase of 80:20 hexane/dichloromethane, flow rate of 4 mL/min for 1 h, discarding initial 18 min. of eluent). The resulting solution is rotary evaporated to approximately 5 mL, then solvent exchanged with isooctane to a volume of 1 mL for dosing to fish cells and measurement of ethoxyresorufin-O-deethylase (EROD) activity.

SPMD extracts are tested for EROD induction potency in *Poeciliopsis lucida* hepatoma cells (PLHC-1). The PLHC-1 bioassay procedures are a slight modification of the H4IIE bioassay methods (Tillitt *et al.* 1991) adapted for 96-well microtitre plates as described in Tysklind *et al.* (1994) in which EROD activity is determined fluorimetrically. The PLHC-1 cells are dosed for 72 h with sample extracts or standards in isooctane or dimethylsulfoxide (DMSO). EROD activity of the samples is calibrated against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for the determination of TCDD-equivalents (TCDD-EQ) in the samples. Potencies, expressed as TCDD-EQ (pg/g SPMD), are calculated based on the whole weight of the SPMD (5 g) as the polyethylene membrane and the triolein both contain compounds.

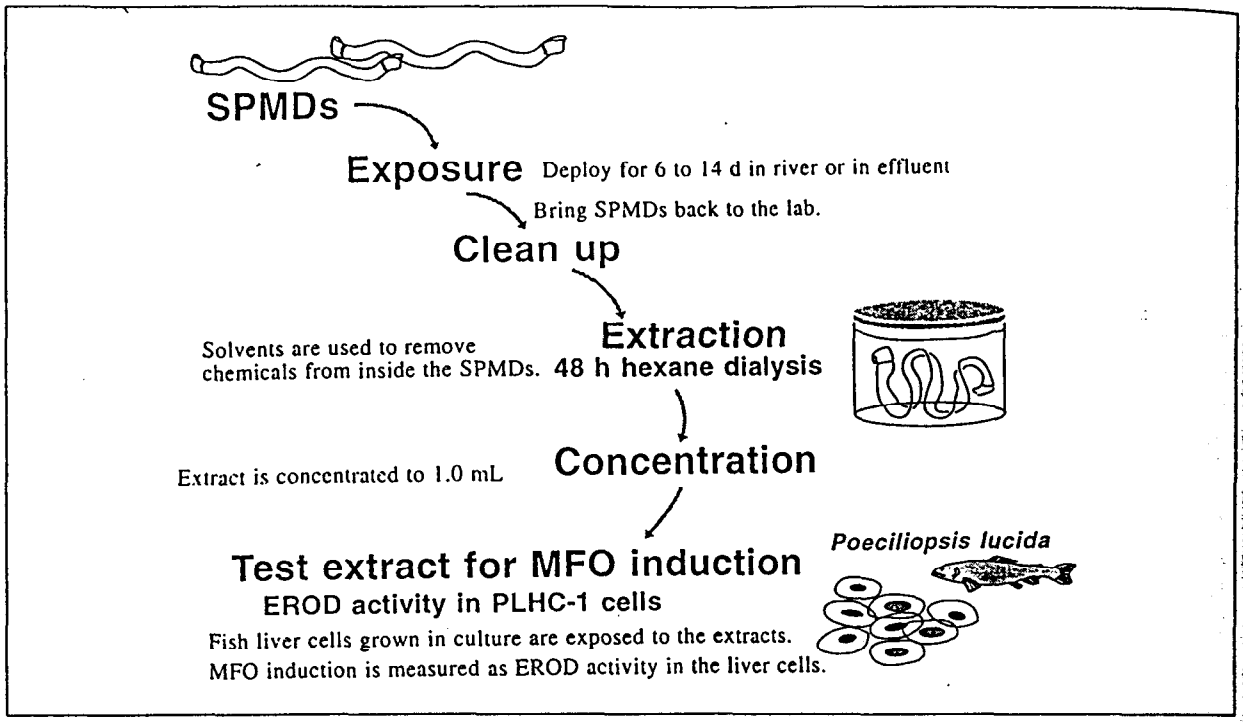

Figure 1. Schematic representation of methods of exposure, extraction and testing of SPMD extracts for MFO induction in fish liver cells.

The expression of potency of the SPMD extracts as pg TCDD-EQ/g does not imply that the SPMDs contained TCDD. Rather, the compounds accumulated by the SPMDs have the equivalent EROD inducing potency as a certain amount of TCDD in the PLHC-1 cells.

Field and Laboratory SPMD Exposures

Field Exposure - bleached kraft pulp mill, Jackfish Bay, Ontario

This study assessed the viability of SPMDs as concentrators of MFO inducers from pulp mill effluent under field conditions. SPMDs accumulated chemicals from an effluent flowing into Jackfish Bay, Lake Superior. SPMDs deployed for 6 days in the outfall of the secondary treatment pond had 284 and 2,665 pg TCDD-EQ/g SPMD (Parrott *et al.*, 1994). The widely different potencies of replicate SPMDs deployed in the mill outfall could be due to problems with the exposures. The foam raised one of the SPMD deployment devices above the effluent, so these SPMDs may not have been exposed to effluent the whole time. Downstream of the effluent ponds (5 to 15 km), SPMDs extracts contained 115 to 268 pg TCDD/g SPMD (Parrott *et al.*, 1994). The pulp mill effluent was known to induce MFO both in wild fish (Munkittrick *et al.*, 1991) and fish caged on site (Munkittrick *et al.*, 1996). Fish caged for 3 days in effluent (5 km downstream) showed 10 to 100 fold increase in EROD activity (Parrott, unpublished data). SPMDs were able to concentrate inducers from various sites on the effluent stream. The large variability of duplicate SPMDs placed in the effluent pond emphasizes the need for careful deployment. Unexpected happenings can compromise samples or entire studies, thus highlighting the need for replication.

Field exposures - pulp mill effluents and oil sands wastewater, Athabasca River, Alberta

SPMDs deployed for 14 days (during August and September, 1994) in waters of the Athabasca River and in effluents from four pulp mills and one oil refinery accumulated chemicals that induced MFO in the PLHC-1 cell line (Parrott *et al.*, 1996a). Extracts of SPMDs from pulp mills were two to five times as potent as extracts of SPMDs exposed to background river water (Figure 2). SPMD extracts from three pulp mill effluents had 62.0, 53.5, and 29.7 pg TCDD-EQ/g, significantly more than in Athabasca River water (12.6 pg TCDD-EQ/g = "background"). SPMDs exposed to effluent from a fourth mill (Pulp mill M, Figure 2) had potencies within the 95 % confidence interval of background. The concentrations of MFO inducers in SPMDs exposed to river water increased downstream of Fort McMurray (58.5 to 728 pg TCDD-EQ/g) and SPMDs deployed in effluent from the oil sands mining and refining facility accumulated the most MFO-inducing chemicals (16,800 pg TCDD-EQ/g, Figure 2). SPMD accumulation in the oil sands area was highly variable, which

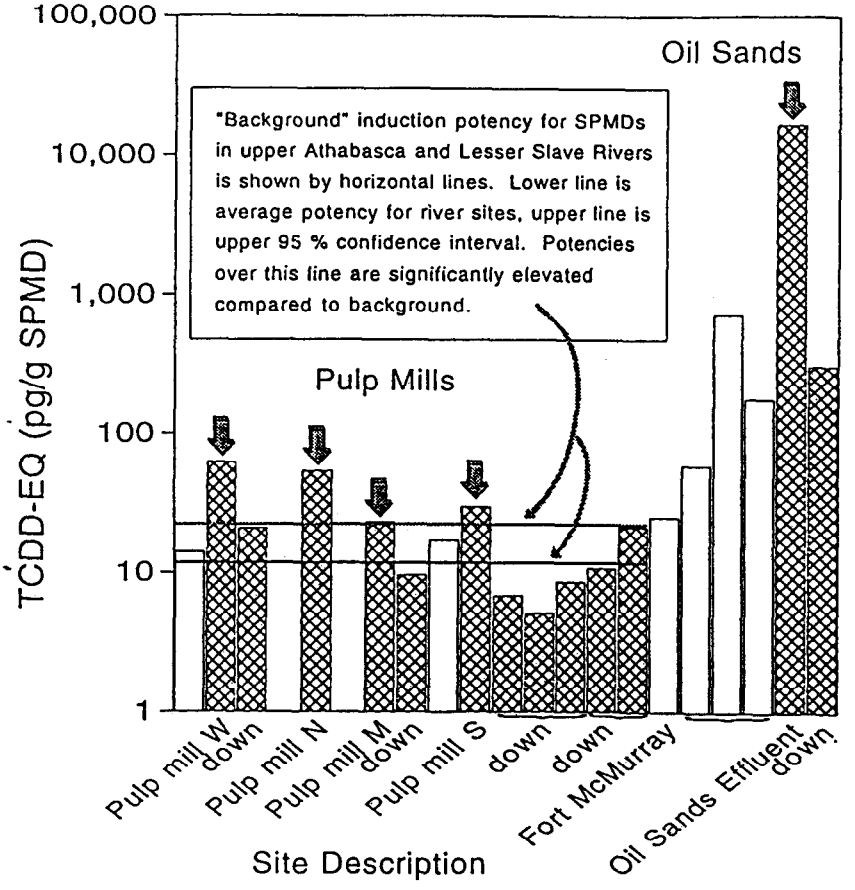

Figure 2. Potency of SPMD extracts (TCDD-EQ, pg/g SPMD) for MFO induction in fish liver cells. SPMDs were deployed for 14 days (August and September, 1994) at sites on the Athabasca and Lesser Slave Rivers and in effluents from pulp mills and an oil sands mining and refining facility. Open bars are upstream sites and hatched bars are effluents and downstream ("down") sites. It should be noted that in the follow-up field study in 1995, there were two sites on Athabasca River tributaries in the oil sands area that had MFO-inducing potencies as high as the oil sands mining and refining effluent. Modified from Parrott *et al.*, 1996a.

suggested an unknown source of inducers, possibly input from natural erosion of the tar sands or another unidentified industrial or municipal source. Although this study was preliminary, the results suggested the four pulp mill effluents contained small quantities of MFO inducers. By contrast, very high quantities of MFO inducers were detected in the oil refinery effluent. Also, high levels of inducers were seen at several Athabasca River sites upstream of the oil sands area. Repeat exposures of SPMDs in the summer of 1995 showed similar results, with the oil sands mining and refining wastewaters containing high levels of MFO inducers (Parrott *et al.*, 1996b). The 1995 sampling included several more sites around the oil sands. Two sites on the mouths of tributaries flowing into the Athabasca R. were discovered to have levels of MFO inducers close to that of the oil sands effluent. These SPMD samples showed that seepage from or weathering of the oil sands can result in naturally high levels of MFO inducers in waters of the Athabasca River.

The Athabasca SPMD studies showed the value of SPMDs under high water velocities, where fish caging would be difficult. It also shows the sensitivity of the fish cell line MFO assay for detecting inducing compounds, as SPMD-extracts from all river sites induced MFO to some extent.

Field exposures - refinery effluent, Mackenzie River, Norman Wells, Northwest Territories

SPMDs deployed for 11 to 12 days in waters of the Mackenzie River and in oil refinery effluent concentrated MFO inducers to levels over 30,000 pg TCDD-EQ/g SPMD (Parrott, unpublished data). Upstream SPMDs contained similar levels of inducers as trip blanks, while downstream (0.5 km) SPMDs contained one-thirtieth the levels of effluent-exposed SPMDs. Induction maxima for refinery effluent exposed SPMDs were about 200 pmol/mg/min, which was one quarter to one half that of TCDD. The observation of potent inducers in SPMDs was in contrast to the finding of only slight induction in fish exposed to effluent in the lab. Small rainbow trout exposed to the refinery wastewater for 3 days showed only a three-fold, non-significant increase in EROD activity compared to control fish. The difference could be due to changes in the refinery effluent; fish were exposed to effluent collected as a single grab sample in June, 1995, while SPMDs were exposed in September 1995 for 11 days. The short exposure time (3 d) of fish could also be insufficient to induce MFO, although for potent bleached kraft mill effluents, induction is detected after 3 days (Munkittrick *et al.*, 1996). Live rainbow trout may have been able to metabolize the refinery effluent inducers and thus render them non-active. This study shows the value of SPMDs as a concentrating device, as during the 11 days of effluent exposures, SPMDs were able to concentrate high levels of inducers from refinery effluent.

Field exposures - sulphite pulp mill and paper mill effluent, Saint John River, New Brunswick

The Saint John River provided an ideal place to study inducers from effluent of a bleached sulphite mill (in New Brunswick, Canada) and effluent of a paper mill (across the river in Maine, USA). Prior effluent studies showed the plumes from each outfall hugged the river sides for over 20 km downstream. SPMDs were deployed for 14 d at two upstream sites and in effluent and three downstream sites on both the NB and Maine sides of the river. In addition, SPMDs deployed in several locations within the pulp mill concentrated inducers that were produced at various stages of pulp production. Induction potencies of SPMD extracts ranged from non-detectable to over 8,000 pg TCDD-EQ/g SPMD. No induction was seen in SPMDs exposed to effluent from the hypochlorite bleaching stage or from the extraction stage of the sulphite process. The most potent SPMD extracts

were from SPMDs deployed in mill effluent prior to clarifiers and secondary treatment ponds. SPMDs in the secondary treatment ponds contained up to 700 pg TCDD-EQ/g SPMD. Downstream of the effluent outfall (0.5 to 5.5 km) SPMDs concentrated 200 to 300 pg TCDD-EQ/g SPMD. SPMDs from sites far downstream (about 15 km from the outfall) had EROD induction potencies similar to upstream sites (non detectable to 100 pg TCDD-EQ/g SPMD). SPMDs deployed in the final effluent from the paper mill had induction potencies similar to background levels upstream and downstream of the paper mill outfall.

The pulp mill effluent contained more inducers than paper mill effluent, and the secondary treatment ponds of the pulp mill were effective at reducing the levels of inducers. Extracts of SPMDs exposed to the secondary treatment effluent were ten to twenty times less potent than those exposed to pre-treated effluent. Downstream of the pulp mill outfall the levels of MFO inducers were two to three times higher than background, but were reduced to background levels at far downstream sites (15 km downstream).

This study represents the first attempt at deploying SPMDs within the pulp mill process facility to determine the levels of inducers present at several stages of pulp processing. The use of SPMDs within process facilities appears useful, but several aspects should be examined more closely. It is unknown whether the severe conditions (60 °C, pH 10) found in many of the in-plant process streams may affect uptake of inducers by the membranes.

Lab Exposure - bleached kraft pulp mill effluent

SPMDs accumulated inducers from barrels of pulp mill effluent held in the laboratory. EROD activity in PLHC-1 cells rose in response to increasing doses of SPMD dialysates. Inducers accumulated by five SPMDs during the six day exposure were similar in potency to 400-500 pg TCDD/g SPMD (Parrott *et al.*, 1994). Reactive clean-up of extracts through a H_2SO_4/silica column removed all EROD inducing potency, suggesting the inducer(s) was not a dioxin or furan-type of compound, as these chemicals would have passed through the H_2SO_4/silica column. Destruction of the inducer by the H_2SO_4/silica column suggests a more labile type of inducer, such as a PAH or natural plant product.

This study showed the possibility for manipulation of SPMD extracts and re-determining potencies as a test procedure to classify types of inducting chemicals. The toxicity identification and evaluation (TIE) approach has not been used, to our knowledge, for MFO inducers using SPMD extracts. TIE has been successful at isolating inducing compounds from pulp mill effluents (Burnison *et al.*, 1996) and from pesticide formulations (Hewitt *et al.*, 1996). SPMDs have been used in TIEs to isolate possible fish tainting compounds from pulp mill effluents (Rohr *et al.*, 1996), so their future use in a MFO-directed TIE appears promising.

Discussion

SPMDs are gaining use as concentrating devices for biological assays, as well as for chemical analyses. The use of SPMDs as concentrating devices for biological testing is relatively new, although research is expanding the types of tests used and applications of the technique. SPMDs have successfully concentrated toxicants and mutagens from urban stream water, Antarctic sediments and PAH-spiked sediments for bioassays with Microtox® and Mutatox® (Johnson 1995; Johnson *et al.*, 1995; Huckins *et al.*, 1996). Huckins *et al.* (1995b) have used SPMDs to concentrate organic chemicals from marine sediments for toxicity tests on *Mysidopsis bahia* and *Ampelisca abdita*. Recently, SPMD extracts from the Detroit River and Lake Erie have been used for SOS-chromotest, *Daphnia magna* lethality tests and Japanese medaka (*Oryzias latipes*) embryo toxicity tests (Metcalfe *et al.*, 1995).

As concentrators of MFO inducers, SPMDs appear promising as their membrane selectivity coincides with the properties of known MFO inducers: planar, non-charged, neutral organic compounds. Extracts of SPMDs could be used in future for bioassay-directed TIEs. SPMD extracts fractionated on HPLC could be dosed to fish cells to determine fractions containing inducing compounds. The fish cell EROD bioassay is beneficial in this approach, as very small volumes (2.5 - 5.0 µL) are needed to dose the fish liver cells.

The dose-response curve describing fish cell EROD activity at increasing concentrations of SPMD extract can give clues as to the nature of the MFO inducers within the SPMDs. Often, in the previous case studies, the EROD maxima for effluent-exposed SPMDs was less than one third of the TCDD maxima. Tests of induction potency and induction maxima for dioxins, furans and PAHs show that for the latter class of compounds, the EROD maxima is lower than for dioxins and furans (Don Tillitt, unpublished data). It is possible that induction maxima for PAHs are lower due to more rapid metabolism and excretion of these compounds, whereas the dioxins and furans are more persistent and induce the system fully. The observation of lower EROD maxima for the pulp mill exposed SPMD extracts lends support to the theory that the inducing chemical is similar to a PAH-type of compound rather than to a dioxin-type of compound.

SPMDs have successfully concentrated MFO inducers from "potent" sources such as refinery effluents and pulp mill effluents, as well as from more dilute sources, such as river and creek waters. The provision of a time-integrated sample, as well as the use of the membranes in hostile environments assures their future use as environmental samplers. Rather than replacing fish caging or exposures, the SPMDs are a novel method to be used alongside existing sampling techniques to allow us to gain added information about the sources, levels and nature of inducing compounds in waters and effluents.

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