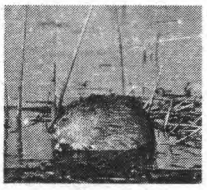
Canada Alberta Northwest Territories

Northern River Basins Study



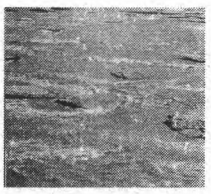


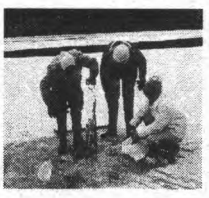





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 127

ACCUMULATION OF FISH MIXED FUNCTION OXYGENASE INDUCERS BY SEMIPERMEABLE MEMBRANE DEVICES IN RIVER WATER AND EFFLUENTS, ATHABASCA, PEACE AND WAPITI RIVERS, AUGUST AND SEPTEMBER, 1995



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by

J. L. Parrott, J. J. Whyte, S.M. Backus and M. E. Comba
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NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 127

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PREFACE:

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

It is explicit in the objectives of the Study to report the results of technical work regularly to the public. This objective is served by distributing project reports to an extensive network of libraries, agencies, organizations and interested individuals and by granting universal permission to reproduce the material.

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Whereas the above publication is the result of a project conducted under the Northern River Basins Study and the terms of reference for that project are deemed to be fulfilled,
IT IS THEREFORE REQUESTED BY THE STUDY OFFICE THAT;
this publication be subjected to proper and responsible review and be considered for release to the public.

F. J. Wrona — 14 May 96
(Dr. Fred J. Wrona, Science Director) (Date)

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
IT IS HERE ADVISED BY THE SCIENCE ADVISORY COMMITTEE THAT;
this publication has been reviewed for scientific content and that the scientific practices represented in the report are acceptable given the specific purposes of the project and subject to the field conditions encountered.
SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No

P. A. Larkin — 24 May 96
(Dr. P. A. Larkin, Ph.D., Chair) (Date)

Whereas the Study Board is satisfied that this publication has been reviewed for scientific content and for immediate health implications,
IT IS HERE APPROVED BY THE BOARD OF DIRECTORS THAT;
this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

Lucille Partington — May 29/96
(Lucille Partington, Co-chair) (Date)

Robert McLeod — May 21/96
(Robert McLeod, Co-chair) (Date)

ACCUMULATION OF FISH MIXED FUNCTION OXYGENASE INDUCERS BY SEMIPERMEABLE MEMBRANE DEVICES IN RIVER WATER AND EFFLUENTS, ATHABASCA, PEACE AND WAPITI RIVERS, AUGUST AND SEPTEMBER 1995

STUDY PERSPECTIVE

The aquatic fauna of the Peace, Athabasca and Slave rivers are exposed to pulp mill effluent, and other types of industrial and municipal discharges. To understand the risks to fish from industrial effluent discharged into these rivers, it is important to know the distribution and fate of chemicals in receiving waters, i.e., the sites of contamination, and the biological responses of fish. Mixed function oxygenase (MFO) induction in fish liver is one of the easier and more sensitive responses to detect. It has been adopted in a wide range of environmental monitoring programs as the primary step preceding any detailed investigations. Briefly, MFOs are liver enzymes that increase after exposure to certain environmental stressors (e.g., PCBs, PAHs, dioxins and furans). Increased MFO activity is frequently observed in fish sampled from waters containing pulp mill effluent and is often associated with other changes in reproduction, growth, pathology and physiology. However, intensive sampling of fish for physiological analyses from one site can be detrimental to that fish population, and is costly. New technology has been developed in the form of semipermeable membrane devices (SPMDs), which act as surrogate fish by absorbing contaminant compounds. In a preliminary investigation conducted in 1994, extracts from SPMDs set in pulp mill effluents on the Athabasca River exhibited higher levels of MFO induction than SPMDs in river water when exposed to live fish cells. The most potent inducers were found in SPMDs from the river near Fort McMurray and the effluent from Suncor. A follow-on study was undertaken to (1) re-examine the variable results from the oil sands area, (2) sample effluent from pulp mills not examined in 1994, and (3) test the response of life fish to the Suncor effluent.

Related Study Questions

1a)	*How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?*
4a)	*What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?*
13b)	*What are the cumulative effects of man-made discharges on the water and aquatic environment?*

This study used SPMDs to identify industrial effluents that induce MFO activity in fish cell lines. SPMDs were deployed for two weeks at three bleached kraft pulp mills and one oil sands facility, and two tributaries near Fort McMurray (Clearwater and Steepbank rivers). The effluents sampled included Weyerhaeuser Canada on the Wapiti River, Daishowa-Marubeni International) on the Peace River, and Alberta-Pacific Forest Industries and Suncor on the Athabasca River. SPMDs provided samples of known exposure time in effluents and river waters by simultaneously sampling the effluent stream and the river upstream and downstream of the mixing zone at each site. In addition, laboratory experiments were conducted with small rainbow trout to quantify the MFO response in fish exposed to various concentrations of the Suncor effluent.

Extracts of SPMDs from the three pulp mills were not significantly different in MFO inducing potency than SPMDs exposed to background river water. The levels of MFO inducers in SPMDs exposed to Athabasca River water increased downstream of Fort McMurray. Extracts of SPMDs from the Suncor refinery wastewater, Steepbank River and Clearwater River all showed MFO induction potencies that were more than 10 times higher than river water upstream of Fort McMurray. SPMDs absorbed a complex mixture of hundreds of compounds, making it difficult to discern the MFO inducers, but high concentrations of PAHs were found in SPMDs from the Suncor effluent, Steepbank and Clearwater rivers. A 96 hour laboratory

exposure of small rainbow trout to the Suncor effluent found that MFO induction was significantly higher than control fish when effluent concentrations were above 2%. Compared to exposures of live fish, the SPMD/fish cell line technique seemed less sensitive to the Suncor effluent.

Similar to the preliminary study, results from this project indicate that SPMDs from three pulp mill effluents contained relatively small quantities of MFO inducers. The high concentrations of PAHs in SPMDs from the Suncor effluent and two tributaries likely contributed to the higher MFO inducing potency in the oil sands area. This new technology is a promising tool for studying effects on aquatic environments. These results will be linked to data obtained with other techniques and measures used by NRBS, enabling a more complete understanding of ecological integrity and health in these rivers.

REPORT SUMMARY

Semipermeable Membrane Devices (SPMDs) were deployed for 2 weeks (August to September, 1995) in waters of the Athabasca, Peace and Wapiti Rivers and in three pulp mill effluents and wastewater from one oil sands mining and upgrading facility. Success of recovery of the SPMDs was 98 %. However, only 80 % of the SPMDs were useable as rapidly dropping water levels after deployment resulted in several SPMDs being exposed to air.

SPMD extracts accumulated chemicals that induced mixed function oxygenase (MFO) in a fish cell line. For expressing the potency of SPMD extracts as inducers in fish cells, MFO induction in cells exposed to SPMD extracts was compared to MFO induction in cells exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This does not imply that the SPMD extracts contained TCDD or any other dioxin or furan, only that the extracts contained chemicals that were equivalent in MFO-inducing potency to a certain amount of TCDD. MFO induction was expressed as ethoxyresorufin-O-deethylase (EROD) potency equivalents in ng per g SPMD (ng EROD-EQ /g).

Extracts of SPMDs from pulp mills were equipotent to extracts of SPMDs exposed to background river water. SPMD extracts from the three pulp mill effluents (Weyerhaeuser Canada Limited, Daishowa - Marubeni International, and Alberta Pacific) contained 2.2, 5.4 and non-detectable ng EROD-EQ/g, respectively, not significantly different than potencies of MFO inducers in SPMDs from waters of Athabasca, Peace and Wapiti Rivers (< 10 ng EROD-EQ/g = "background") in the area of the pulp mills.

Around the area of Fort McMurray and Suncor Inc., SPMDs were positioned at eight sites on the Athabasca River, or on tributaries such as the Clearwater and Steepbank Rivers, as well as in wastewater of Suncor. The concentrations of MFO inducers in SPMDs exposed to river water increased downstream of Fort McMurray. In this area, SPMDs accumulated inducers from the river at concentrations from 20 to 33 ng EROD-EQ/g. SPMDs from two sites, the south side of the Clearwater River and the north side of the Steepbank River had high induction potencies, 655 and 429 ng EROD-EQ/g, respectively. SPMD accumulation was highly variable in the oil sands area, which indicated an unknown source of inducers, possibly input from natural erosion of the oil sands.

SPMDs deployed in effluent from Suncor accumulated the most MFO-inducing chemicals (358 to 860 ng EROD-EQ/g), with induction potency over ten times that of most SPMDs from river water upstream of Suncor, except for the high potencies of Clearwater and Steepbank River SPMD extracts, which were very close to potencies of Suncor extracts.

SPMD extracts that induced MFO contained many polyaromatic hydrocarbons (PAHs) and C1 to C3-substituted PAHs. SPMD extracts from the Clearwater and Steepbank Rivers showed high concentrations of acenaphthylene, acenaphthene, fluorene, phenanthrene and methyl and dimethyl phenanthrene/anthracenes, compared to Suncor-SPMDs. Conversely, Suncor wastewater SPMD extracts contained higher concentrations of pyrene than SPMDs from the Steepbank or Clearwater Rivers. It is unknown which, if any, of the PAHs detected in the SPMDs were causing the MFO

induction seen in fish cells exposed to the SPMD extracts.

A 96 h laboratory exposure of small rainbow trout to Suncor wastewater resulted in induction. Maximum induction was seen at exposures of fish to 32 % effluent, and induction was as high or higher than fish exposed to the positive control, 10 μg/kg B-naphthoflavone (BNF). EROD induction was significantly higher than control fish at effluent concentrations above 2 %, and was as high as BNF at wastewater concentrations of 20 %.

Live fish exposed to wastewaters and the fish cell line exposed to SPMD extracts of wastewaters appear equally sensitive in their MFO responses. Although the induction maxima for MFO in fish cells was low, very minute quantities of Suncor SPMD extracts were required to produce a response.

Although this study was preliminary, the results indicated that SPMDs from the three pulp mill effluents contained very low quantities of MFO inducers. Compared to MFO induction by extracts of SPMDs deployed in four pulp mills on the Athabasca and Lesser Slave Rivers in the summer of 1994, these three pulp mill effluents were also very low in potency. SPMDs deployed in Weyerhaeuser, Daishowa, and Alberta Pacific were much less potent than SPMDs from two Ontario bleached kraft mill effluents. By contrast, high quantities of MFO inducers were accumulated from Suncor effluent, and from two sites on the Athabasca River in the oil sands area, the south side of the Clearwater River and the north side of the Steepbank River, indicating some unknown anthropogenic or natural source in this area.

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1.0 INTRODUCTION

The purpose of this study was to identify effluents (from three pulp mills and one oil sands mining and upgrading facility along the Athabasca, Peace and Wapiti Rivers) that contained compounds that induce mixed function oxygenase (MFO) activity in fish. We also wished to determine the influence of natural seeps from the oil sands area as contributors to the MFO inducers in the Athabasca River.

1.1 Mixed Function Oxygenases (MFOs)

Mixed function oxygenases (MFOs) are liver enzymes in fish that increase after exposure to certain compounds. The increase in MFO activity usually indicates an increase in the amount of enzyme in the liver cells and is referred to as induction (Okey, 1990). Increased 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). 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), co-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 fish exposed to pulp mill effluent is often found along with other changes in fish reproduction, growth, pathology and physiology, it is important to know the nature and concentration of compounds affecting the MFO system.

1.2 SPMDs

Semipermeable membrane devices (SPMDs) are layflat polyethylene membrane tubes containing a thin film of purified triolein, a substance that constitutes a major fraction of the neutral lipid of fish. SPMDs were developed by Huckins *et al.* (1990) as a passive *in-situ* sampler that, when immersed in water, absorbs water insoluble chemicals with a molecular weight of about 600 or less. Freely dissolved neutral organic chemicals diffuse through pores in the polyethylene membrane and dissolve in the triolein. The passage of chemicals through the membrane pores of an SPMD simulates the diffusion of compounds across a live fish gill membrane. The concentration of hydrophobic compounds in the membrane and triolein of an SPMD is similar to the process by which fish take up and store waterborne neutral organic chemicals (Huckins *et al.* 1990), allowing SPMDs to be used as surrogate fish. SPMDs offer a sampling technique that permits the lipid to be analyzed by traditional chemical techniques and by bioassays to estimate concentrations of compounds with specific bioactivity (eg. inducers of the mixed function oxygenases).

SPMDs provide time-integrated samples of effluents and river waters. This allows a representative sample to be gathered, which will be less vulnerable than a single sample to changes due to pulses of chemicals and different processes within the pulp mills or oil sands upgrading facilities.

Another advantage of SPMDs is one of logistics. SPMDs can be made to any size (usually, 91 cm long,

2.5 cm wide and 0.1 cm thick), can be shipped by mail and can be deployed from shore, by wading or from any size boat. In laboratory studies with these devices, SPMDs were found to accumulate inducers from pulp mill effluent, as shown by bioassays of extracts using fish cells in culture (Parrott *et al.* 1994). The cell line results were considered to be as good a detector of these compounds as whole fish. The use of these devices in the field requires much less effort than would caged fish or exposure of laboratory fish to stream water shipped to the lab.

The disadvantages of SPMDs relate to the selectivity of the membrane: only freely dissolved neutral organic compounds are sampled. While this selectivity is similar to that of a fish membrane, the SPMDs lack the active and facilitated transport processes of a living membrane. Charged ions (metals such as Cu^{++} and Zn^{++}, or ionized phenols and acids) are not taken up, as there is resistance of passage through the neutral polymer membrane. Another difference between SPMDs and fish is that the SPMDs cannot metabolize the compounds. While this is an advantage for analytical detection, it must be recognized the compounds accumulated by SPMDs may not be accumulated by biological organisms to the same extent, as the organisms may have the ability to break down and excrete the chemicals. Also, SPMDs can mimic only the waterborne uptake of chemicals into an organism. If the foodchain is the main route of uptake of a chemical, SPMDs will not predict bioaccumulation.

SPMDs had been deployed in the summer of 1994 at several sites along the Athabasca and Lesser Slave Rivers and in four pulp mill effluents (Weldwood, Alberta Newsprint and Slave Lake Pulp and Millar Western) and in Suncor wastewater (Parrott *et al.* 1996). Induction in fish cells exposed to SPMD extracts showed low background levels of inducers in Athabasca River water from Hinton to Boyle. SPMDs from pulp mill effluents surveyed in 1994 were two to five times as potent as background. In the area downstream of Fort McMurray, SPMDs in Athabasca River contained potent inducers, suggesting natural seeps from the oil sands or from anthropogenic input from the town. SPMDs from Suncor contained the highest levels of MFO inducers.

In the second year of the study, we wanted to examine SPMDs from three pulp mills that were not included in the 1994 survey, Weyerhaeuser Canada Limited, Daishowa - Marubeni International, and Alberta Pacific. In addition, we wanted to investigate more closely the variability in inducers in the area of the oil sands. To do this, eight sites were chosen to deploy SPMDs in the oil sands area, as well as deployments in wastewater to repeat the 1994 sample.

Because very high levels of inducers were detected in SPMDs from Suncor wastewater in the 1994 survey, we also wanted to test the response of live fish to this wastewater. Wastewater was collected on two occasions, and shipped to NWRI where exposures of fish began within three days of water sampling. Small rainbow trout were exposed to graded concentrations of the wastewater for 4 d, after which their liver EROD activities were measured.

2.0 MATERIALS AND METHODS

2.1 Site Descriptions

All SPMD deployment sites were on the Athabasca, Peace and Wapiti Rivers (Figure 1). Three pulp mills and one oil sands mining and upgrading facility were chosen for the study and SPMDs were deployed in the final effluents. Upstream sites on the rivers were 2 to 9 km above and downstream sites were 4 to 5 km below the pulp mills. For the oil sands facility, more detailed deployments were done, ranging from 34 km upstream to 27 km downstream of the wastewater outfall (Table 1). Some far downstream sites were chosen to determine influence of merging rivers (Clearwater and Steepbank Rivers) or cities (Fort McMurray). Latitude and longitude of each river deployment site is given in Table 2.

SPMDs were installed in duplicate upstream and in duplicate or triplicate downstream of each source and in effluent treatment ponds, plus several 'far-field' sites (Table 1). A total of 45 sites were sampled. Two deployment devices, each containing two SPMDs, were used at upstream sites, two deployment devices, each containing three SPMDs, were used inside the pulp mills and oil sands facility, and two or three replicate deployment devices, each containing two SPMDs, were used at downstream locations. SPMDs were deployed in the pulp mill's secondary treatment ponds where the effluent was leaving the ponds. In the oil sands mining and refining facility, SPMDs were deployed in the effluent just before it merged with a cooling water stream prior to discharge to the river. At each site, 2 SPMDs were used as trip blanks, and were exposed to air, handled as if deployed, and returned to the sealed can. The sampling was done on the declining hydrograph during August 13 to September 7, 1995, at water temperatures between 12 and 17 °C.

2.2 Sampling Equipment

SPMDs

SPMDs were purchased from Environmental Sampling Technologies (St. Joseph, Missouri). SPMDs were 91 cm long x 2.5 cm wide low density polyethylene layflat tube (wall thickness 0.80 μm) filled with 1 mL (0.915 g) high purity (95 %) synthetic triolein. SPMDs, sealed in tins, were sent to NWRI labs.

SPMDs were shipped at ambient temperatures from NWRI labs to the field site. Several precautions were taken to prevent contact of SPMDs with contaminated field equipment. One person deployed the SPMD and handled only the deployment device and the SPMDs, while the other person controlled the boat. Gloves were used while handling SPMDs and deployment devices. The deployments were performed as quickly as possible to reduce exposure to air and contaminants during handling. Trip blank SPMDs were open to the air for the same amount of time and were handled in the same manner as deployed SPMDs. When deployments were finished, trip blanks were returned to the sealed cans.

Deployment Devices

From the experiences of the 1994 Athabasca SPMD field trip, it was decided that stronger deployment

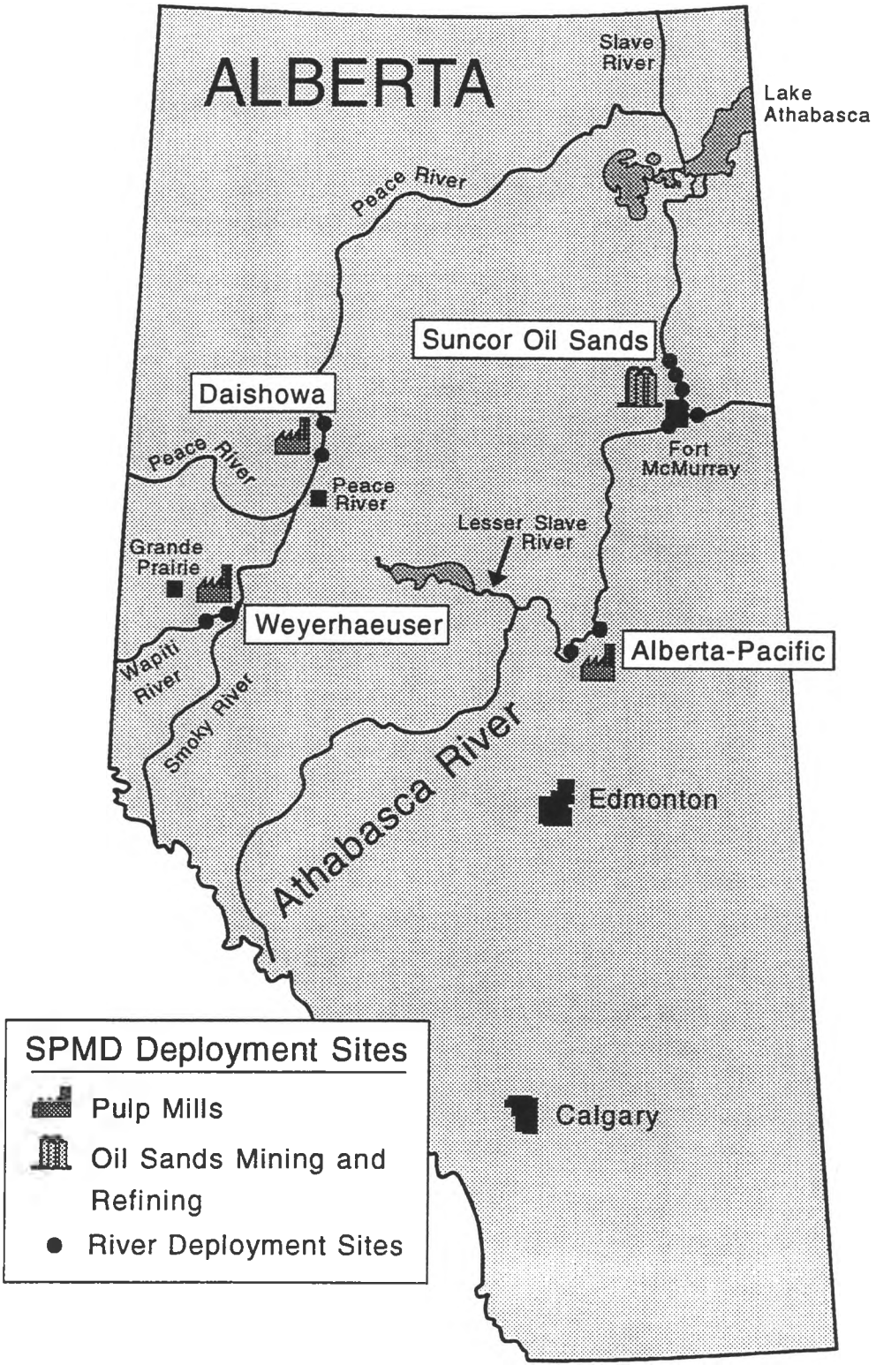

Figure 1. Location of SPMD deployment sites, August to September, 1995.

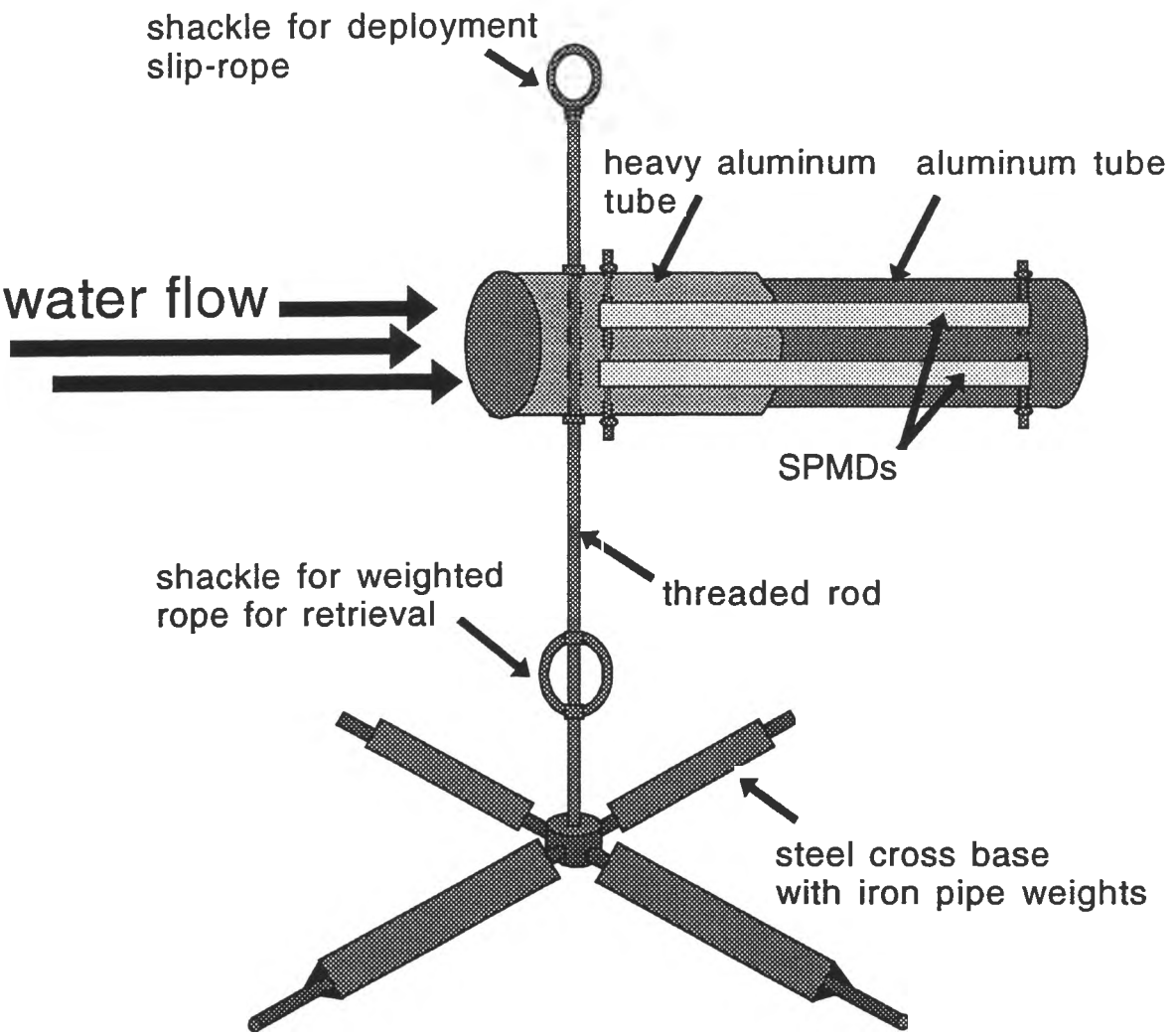

Figure 2. Deployment device used to hold SPMDs in position at sites on the Athabasca, Wapiti and Peace Rivers, August to September, 1995.

Table 1: Codes for sampling sites and number of SPMDs deployed in pulp mill effluents and oil sands wastewater and at upstream and downstream sites on the Athabasca, Wapiti and Peace Rivers, August to September, 1995.

Code	Distance downstream (km)	Location	SPMDs	Total	SPMD Blanks
Alberta-Pacific					
upstream Alberta Pacific	- 3.2	upstream	2 x 2	4	2
Alberta Pacific	0	in mill treatment pond	2 x 2	4	2
downstream Alberta Pacific	5.2	downstream from	2 x 2	4	2
Fort McMurray / Suncor / Oil Sands Area					
upstream Fort McMurray	- 34.4	upstream of Fort McMurray	3 x 2	6	2
Clearwater River	- 0.8[a], - 31.2[b]	mouth of Clearwater R.	2 x 2	4	2
downstream Fort McMurray	- 17.0	downstream of Fort McMurray	3 x 2	6	2
upstream Suncor	- 2.0	upstream of Suncor	3 x 2	6	2
Suncor	0	in final wastewater pond	3 x 2	6	2
Steepbank River	0.4[a], 2.4[b]	mouth of Steepbank River	2 x 2	4	2
downstream near Suncor	4.0	downstream of Suncor	3 x 2	6	2
downstream far Suncor	18.4	downstream of Suncor	3 x 2	6	2
Daphne Island	27.2	Daphne Island	3 x 2	6	2
Weyerhaeuser Pulp Mill on Wapiti River					
upstream Weyerhaeuser	- 2.4	upstream on Wapiti River	2 x 2	4	2
Weyerhaeuser	0	in final treatment ponds of mill	2 x 2	4	2
downstream Weyerhaeuser	5.2	downstream of Weyerhaeuser	2 x 2	4	2
Daishowa Pulp Mill on Peace River					
upstream Daishowa	- 8.8	upstream of Daishowa	2 x 2	4	2
Daishowa	0	in final treatment ponds at Daishowa	2 x 2	4	2
downstream Daishowa	4.2	downstream of Daishowa	2 x 2	4	2
			Grand Total:	**90**	**38**

[a] distance from SPMD deployment site to mouth of river

[b] distance from mouth of river to effluent outfall

Table 2. Latitude and longitude of SPMD deployment sites on Athabasca, Peace and Wapiti Rivers, August to September, 1995.

Station	Side	Latitude	Longitude
upstream Alberta Pacific	south	54 57'17"	112 57'25"
	north	54 57'50"	112 49'50"
Alberta Pacific	pond		
downstream Alberta Pacific	south	54 57'26"	112 50'01"
	north	54 57'50"	112 49'50"
upstream Fort McMurray	west	56 43'15"	111 25'19"
	east	56 43'00"	111 25'17"
	middle	56 43'08"	111 25'18"
Clearwater River	north	56 44'45"	111 22'35"
	south	56 44'40"	111 22'40"
downstream Fort McMurray	east	56 51'48"	111 25'20"
	west	56 52'03"	111 26'08"
	middle	56 51'57"	111 25"42"
upstream Suncor	west	56 58'45"	11126'55"
	east	56 58'45"	111 26'20"
	middle	56 58'45"	111 26'40"
Suncor	pond		
Steepbank River	north	57 01'15"	11128'45"
	south	57 01'12"	111 28'40"
downstream Suncor	west	57 03'48"	11 30'02"
	east	57 03'48"	111 29'30"
	middle	57 03'48"	111 29'48"

Station (cont.)	Side	Latitude	Longitude
far downstream Suncor	east	57 08'13"	111 36'20"
	west	57 08'10"	111 36'35"
	middle	57 08'12"	111 36'30"
Daphne Island	east	57 12'36"	111 36'20"
	west	57 12'52"	111 36'54"
	middle	57 12'40"	111 36'42"
upstream Weyerhaeuser	east	55 03'53"	118 40'40"
	west	55 03'57"	118 40'47"
Weyerhaeuser	pond		
downstream Weyerhaeuser	east	55 04'25"	118 35'28"
	west	55 04'32"	118 35'20"
upstream Daishowa	west	56 21'12"	117 11'50"
	east	56 12'10"	117 11'34"
Daishowa	pond		
downstream Daishowa	west	55 24'27"	117 10'09"
	east	55 24'18"	117 09'50"

devices were necessary to withstand the high flows of the Athabasca, Peace and Wapiti Rivers. Designs were proposed and prototypes were made. Modifications included replacing the patio stone base of the deployment device used in 1994 with a large, heavy steel cross. This prevented the "kite effect" of the patio stone in the current while deploying the device, and still provided a heavy and stable base. A heavy aluminum collar was added to the tube containing the SPMDs. In the original design used in 1994, the aluminum connection wore away in the current, resulting in loss of SPMDs. The modification strengthened the collar joining the SPMD tube and the vertical pole. Also, a thicker gauge aluminum was used for the entire tube.

At each site, the deployment devices and SPMDs were assembled on shore. SPMD deployment devices were constructed of long aluminum tubes with pins at each end to hold SPMDs (Figure 2). Tubes were held 60 to 80 cm off the river bottom by a 1 m threaded steel rod from a 90 cm steel cross base that was weighted with iron pipe. The aluminum tube could be set at any height from the bottom by adjusting nuts on the threaded rod, and the whole tube assembly was designed to rotate freely in the current. A thicker gauge aluminum tube was used for connecting the tube with the threaded rod, to prevent wearing away of the tube as the assembly rotated with the current. SPMDs were suspended lengthwise in a 95 x 10 cm diameter piece of aluminum tube by carriage bolts through the loops at each end of the SPMDs. In this manner the SPMDs were kept separated. At one end of the tube, two 1.4 cm holes were drilled through the tube to accommodate a 1 m length of 1.3 cm threaded rod. The device was lowered with a slip rope. A second rope attached to a shackle on the lower part of the threaded rod was tied to trees or other stable objects on shore. In the river installations, a 25 to 30 m length of weighted rope was left attached to the device and allowed to sink downstream. Devices were retrieved from sampling locations using a grapple hook to drag the river bottom until the weighted rope was recovered. This method was employed to ensure that there were not any visible clues to hunters, fishermen or boaters, that there was a sampling device in the river.

When sampling on the river, SPMD devices were either deployed by lowering the devices into the river from a small boat and once from a bridge over the river. At sites upstream of effluent discharge sources, two deployment devices, each containing two SPMDs, were deployed. For downstream sites, three deployment devices, each containing two SPMDs, were equally spaced in the river on a transect from one bank to the other.

To deploy in mill effluent streams, SPMDs in aluminum tubes were weighted with steel bars and suspended by rope or wire cable in the effluent. All effluent deployments were in flowing channels or treatment ponds, with SPMDs sampling the effluent just before it merged with river water (for the pulp mills) or before it merged with a cooling water stream prior to discharge to the river (for the oil sands site). In effluents, two deployment devices (pulp mills) or three deployment devices (Suncor), each containing two SPMDs, were used.

Exactly two weeks after deployment, SPMDs were retrieved from their locations. After the device was removed from the water, the SPMDs were cut from their carriage bolt supports and immediately placed into empty paint-type cans. SPMDs were sealed in tins and frozen for transport back to the labs at NWRI.

2.3 Water Chemistry

Water velocity, conductivity, temperature and pH
At the same time as SPMDs were deployed and retrieved, river velocity, water temperature, conductivity and pH were measured. There were no water velocity readings for the effluent and wastewater streams as the high electrical conductivity of the effluent shorted out the water velocity meter.

Water velocity measurements were obtained using a Price Model 1210AA velocity meter (Scientific Instruments) while conductivity was measured using a portable Hanna HI 8633 Conductivity Meter. This was calibrated before use by the Calibration Unit of Engineering Services at NWRI. Temperature and pH were measured using a portable Hanna HI 8424 Microcomputer pH Meter. This meter was also calibrated before use by the Calibration Unit and calibration was checked on a daily basis in the field using pH reference standards.

2.4 SPMD dialyses and clean up

SPMD containers were frozen upon return to NWRI. Substances fouling the external membrane surface were removed in a stainless steel container by scrubbing with cold tap water and a toothbrush. Each membrane device was sequentially rinsed with methanol, then hexane and air dried for approximately two minutes on solvent washed tinfoil. Membrane(s) were placed into either 500 mL (1 device) or 1 L (2 devices) glass mason jars, capped and frozen for up to two hours until solvent addition. Each sample jar was consecutively filled to the neck with pesticide grade hexane (approximately 400 mL/ SPMD), and covered with caps lined with solvent washed tinfoil. The jars were dialysed for 48 h in a temperature controlled water bath at 19 °C. The samples were gently agitated every 12 h to improve mixing. After 48 h the SPMDs were removed from the dialysate and discarded.

The dialysate was rotary evaporated to about 5 mL and filtered into a centrifuge tube through a micro column of anhydrous sodium sulphate. The eluent was concentrated to 1 mL using a temperature and pressure controlled nitrogen evaporator (N-EVAP, Organomation, Berlin, MA). Compounds of interest were separated from residual triolein with size exclusion high pressure liquid chromatography (HPLC). An isocratic mobile phase of 80:20 hexane/dichloromethane was employed. The chromatographic column was 250 x 22 mm of phenogel (Phenomenex, Torrance, California) adsorbent. The flow rate of the mobile phase was 4 mL/min for one hour, with the initial 18 minutes of eluent being discarded. The remaining chromatographic solution was rotary evaporated to approximately 5 mL. The concentrates were transferred to centrifuge tubes and solvent exchanged with trimethyl pentane to a volume of 1 mL. A sub-sample of 100 µL was removed for future chemical analyses. The remaining extract was quantitatively transferred into a micro evaporation vial and concentrated to 200 µL for dosing to fish cells for the ethoxyresorufin-O-deethylase (EROD) assay.

2.5 PLHC-1 Bioassay Methods

SPMD extracts were tested for EROD induction potency in *Poeciliopsis lucida* hepatoma cells (PLHC-1). The PLHC-1 bioassay procedures were a slight modification of the H4IIE bioassay methods

described in Tillitt *et al.* (1991). The PLHC-1 cells seeded at 20,000 cells/well in 500 μL of D-MEM culture media in 48-well microtitre plates. After a 24 h incubation, the cells were dosed with sample extracts or standards in a 5 μL volume of isooctane. The cells were exposed to eight different concentrations (doses) of the samples in a 25% dilution series, with three replicates at each dose. The samples were calibrated against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for the determination of EROD potency-equivalents (EROD-EQ) in the samples. TCDD standards were dosed at eight concentrations or doses (0, 12.5, 25, 50, 100, 200, 400, 800 pM) with each dose replicated three times.

A 72 h incubation followed dosing of the cells, after which the plates were washed three times with ultra-pure water and the cells allowed to lyse. The following reagents were added to each well: 20 μL of Tris-sucrose (0.05 to 0.2 M) with dicoumerol (20 μM final concentration) and 20 μL of 5 μM 7-ethoxyresorufin (0.5 μM final concentration). Initial fluorescence in each well was read on the fluorometric plate reader (excitation filter wavelength centred at 530 nm and emission filter wavelength centred at 595 nm, Cytofluor 2300, Millipore Corp.) and the cells returned to the 30 °C incubator and gently agitated. Fluorescence was read after 10 min and the activity calculated by subtraction.

The relative fluorescence intensity of the samples was then compared to a resorufin standard curve and the relative intensity units were converted to pmol resorufin. the data from each well ethoxyresorufin-O-deethylase (EROD) rate (pmol/min). The amount of protein in each well was determined by fluorescamine assay (Lorenzen and Kennedy, 1993) and these values were used to normalize dose to each well and EROD activity. The doses of each sample (dilution of SPMD extract) or TCDD standards (pM TCDD) were plotted against EROD activity (pmol/min/mg cellular protein) to develop dose-response curves. EC50s derived from these curves were used to compare the relative potencies of the samples with that of the standard, TCDD.

Where triphasic curves were found, EC50s were determined from the first part of the curve (the rising EROD activity) with the remaining part of the curve ignored. When the curve comprising the increasing EROD was described by only two concentration points, the EC50 was determined by eye.

The expression of potency of the SPMD extracts as ng EROD-EQ/g does not imply that the SPMDs contained TCDD. Rather, the compounds accumulated by the SPMDs were as potent as, or were equivalent to, a certain amount of TCDD.

Potencies, expressed as EROD-EQ (ng/g SPMD), were calculated based on the whole weight of the SPMD, as the 4 g polyethylene membrane accumulates compounds as well as the 1 g of triolein. The total weight of the SPMD was 5 g. To convert the EROD-EQ ng/g SPMD to ng/g triolein, the ng/g SPMD results would be multiplied by five, giving ng/g triolein (example: EROD-EQ of 200 ng/g SPMD = EROD-EQ of 1,000 ng/g triolein).

2.6 Mass Spectrometry

Sub samples (100 µL) from the 1 mL SPMD dialysate extracts were analyzed by mass spectrometry. Concentrations of polyaromatic hydrocarbons (PAH) were quantified by a HP 5890 gas chromatograph

coupled with a HP 5971 quadrapole mass selective detector (GC-SIM). The helium carrier gas pressure (100 kPa) for the 30 m HP-5MS capillary column was electronically controlled. Sample injections of 2 μl were made with a HP 7673A autosampler. The initial oven temperature was 60 °C for 2 minutes, temperature-programmed to 150 °C at 10 °C/minute, then temperature-programmed to 300 °C at 3.0 °C/minute. The analyses were conducted using electron impact ionization and single ion detection (SIM). The instrument was calibrated to PFTBA using the midmass autotune program. The filament and multiplier were turned on at six minutes. Thirty-six aromatic and alkyl aromatic PAH (Appendix D, Table AD.1) were measured in samples in conjunction with authentic standard retention times and single point molecular ion (M^+) calibration responses.

Full scan electron impact mass spectra were obtained with both the Hewlett-Packard and Varian Saturn capillary column gas chromatograph-mass spectrometers. Full scan (m/e 100 to m/e 550) spectra from the Varian Saturn ion trap quadrapole mass spectrometer were obtained at 70 eV. The Saturn ion trap was coupled directly with the Varian 3400 capillary column gas chromatograph. Two µL of each extract were injected on-column through a septum programmable injector (SPI). A fused silica capillary column (30 M DB5-MS, J&W Scientific) was used for the analyses under the following chromatography conditions: initial temperature of 60 °C for 2 minutes, 10 °C/min to 100 °C; 3 °C/min to 280 °C with a 5 minute hold. The carrier gas was helium. Full scan spectra from the HP mass spectrometer were obtained using the SIM chromatography conditions, except a mass range from m/e 80 to m/e 500 was scanned.

Qualitative characterizations of chemicals in SPMD dialysates were based on interpretation of ion fragments, ion patterns, comparison with library spectra (NBS), comparison with literature references and past experience. Direct analysis of dialysates could only be used for "screening" purposes. Extracts analyzed directly, yielded complex mixtures with many confounding ion fragment series. Consequently, chemical characterizations are "tentative" pending fractionation, clean-up and comparison to specific reference materials. Information, spectra, and criteria used to determine chemical types can be obtained from Comba and Backus, 1996.

2.7 Laboratory Exposures of Fish to Refinery Wastewater

Wastewater from Suncor refinery was tested immediately upon receipt (within 3 d of water sampling) and was stored at 4 °C, and re-tested after one or two weeks. Two batches of wastewater were collected, a Sept 29, 1995 sample was tested on Oct 2-6, 1995 and Oct 16-20, 1995, and an Oct 13, 1995 sample was tested on Oct 16-20, 1995 and Oct 23-27, 1995.

Fish Exposures

Rainbow trout (1-3 g) were exposed to 0.1, 0.32, 1.0, 3.2, 10, 32 or 100 % wastewater concentrations for 4 d. Exposure aquaria were lined with clean plastic bags, and five fish were placed in each tank. Exposure solutions were renewed every 24 h. To control temperature, all tests were carried out in a 15 °C environmental chamber, with 16 h light and 8 h dark photoperiod. Tanks were aerated to maintain dissolved oxygen above 80 % saturation. Water temperature, pH, dissolved oxygen and conductivity were measured daily. Fish were not fed 72 h prior to start of the tests or during the exposures.

At the end of the 4 d exposure, fish were sacrificed by concussion and weighed. Individual livers were dissected, weighed and homogenized in buffer. Liver homogenates were spun (9,000 g for 20 min) and the supernatant (S9) was removed and frozen (-80 °C).

EROD and Protein Assays
Thawed S9 was used for the EROD and protein assay. Buffer, NADPH, cofactors and 7-ethoxyresorufin were mixed, and rate of production of resorufin over 12 minutes was measured in a cytofluor plate reader (Hodson *et al.* 1996). Protein content of the S9 solution was determined by the Biorad spectrophotometric method. Results were expressed as picomoles of resorufin produced per mg of protein per minute (pmol/mg/min).

Positive and Negative Controls
To ensure fish were responsive to inducers, two tanks of 5 fish were exposed to 10 μg/L B-naphthoflavone (BNF), a known inducer of EROD. These fish are referred to as the "positive controls". To determine the natural or constitutive levels of EROD in unexposed fish, two tanks of 5 fish were exposed to water alone (dechlorinated, charcoal filtered Burlington city water). These fish are the "negative controls".

3.0 RESULTS

3.1 Field Notes

River Water Levels
On deployment, river water levels were extremely high due to excessive summer rain in Alberta. Anecdotal evidence from local people suggested river levels were highest they remember. SPMDs were set in up to 3 m of water, and were often tied to overhanging branches of trees.

River levels dropped dramatically between deployment and retrieval of SPMDs. Consequently, several SPMDs were above the water level when retrieved (Table 3, comments). Out of 45 devices deployed, 7 were exposed to air due to dropping water levels. These 14 SPMDs from the 7 air-exposed deployment devices were unusable. Physical recovery of the SPMDs was excellent, with 44 of 45 deployment devices retrieved. However, due to dropping water levels exposing some SPMDs and also to tampering, only 35 of the 44 deployed devices were usable. Thus, 70 SPMDs were returned to the labs for dialyses, cleanup and concentration.

Deployment Devices
The modified deployment device was a great success. Although heavy and cumbersome to assemble and install, these devices were a huge improvement over last year's design. They withstood the high flow of the Athabasca, Peace and Wapiti Rivers, and were in good shape on removal. An additional improvement was the dis-assembleable design: these deployment devices can be re-used on future field trips. The initial costs of engineering and producing these devices for the 1995 trip was several times the cost of the original devices used in 1994, but the investment was worthwhile as we now have a supply of sturdy,

reusable deployment devices.

Lost SPMDs
The only SPMDs unrecovered were those midstream at the site downstream of Suncor (Table 3). At two sites (the site far downstream of Suncor and the site downstream of Daishowa) it appears SPMDs were intentionally removed from the water, as intact deployment devices were found on the river banks. These four SPMDs from the two deployment devices removed from the river are unusable.

3.2 Water Chemistry

Temperature, conductivity, pH and water velocity were taken at each deployment site. All raw data are reported in Appendix B. River water temperatures ranged from about 12 to 17 °C (Figure 3). Effluent temperatures were higher and ranged from about 23 to 33°C. River water pH was relatively constant, about 7.5 to 8.5 and changed little from deployment to retrieval of SPMDs (Figure 4). Conductivity was variable and ranged from 100 to 300 uS, but most values were in the 150 to 200 uS range (Figure 5). Water velocity varied greatly (<0.1 to 1.8 m/sec) at different sites along the rivers, and also varied at different locations (side, midstream, other side) within a site (Figure 6).

3.3 EROD induction in PLHC-1

SPMDs extracts caused EROD induction in PLHC-1 (Table 4), while trip blanks showed little induction (mean potency 1.6 ng EROD-EQ/g, n=24, s.e.= 0.56). Potencies of extracts ranged from a few to over 800 ng EROD-EQ/g (Figure 7a, 7b). All SPMD extracts had lower induction maxima (20-80 pmol/mg/min) than TCDD (maxima of 200-800 pmol/mg/min), and most were not very potent (Table 4). The low induction maxima for the dose response curves is typical of PAH-type inducers in PLHC-1.

Dose response curves varied from classical induction dose response (Appendix C, Figure AC.1, AC.4 top left and middle) to triphasic dose response (Appendix C, Figure AC.4 top right and bottom left and Figure AC.5). Some curves were non-descript, with little induction, so EC50s could not be determined (Appendix C, Figure AC.2 bottom right, Figure AC.3 top left and middle right).

Little induction was seen in background waters of the Athabasca, Peace and Wapiti Rivers (Appendix C, Figure AC.2). In areas of the river upstream and downstream of the pulp mills, all potencies were < 10 ng EROD-EQ/g SPMD. Potencies of extracts from Alberta Pacific and Weyerhaeuser were also low, with most dose-response curves having maxima of <15 pmol/mg/min (Appendix C, Figure AC.3, top and middle). SPMD extracts from Daishowa effluent showed slight induction, with maxima of 20-30 pmol/mg/min (Appendix C, Figure AC.3, bottom). These extracts were not very potent, as EC50s were calculated as 1.6 to 1.8 % extract dilution.

Induction was stronger in cells dosed with SPMD extracts from the oil sands area and the Suncor wastewater effluent (Table 4, Figure 7b). Triphasic dose response curves were most common in SPMD extracts from the oil sands area. Background induction in the oil sands area was 20 to 33 ng EROD-EQ/g, with EC50 s from 0.2 to 0.5 % extract dilution. Dose response curve maxima were 20-80

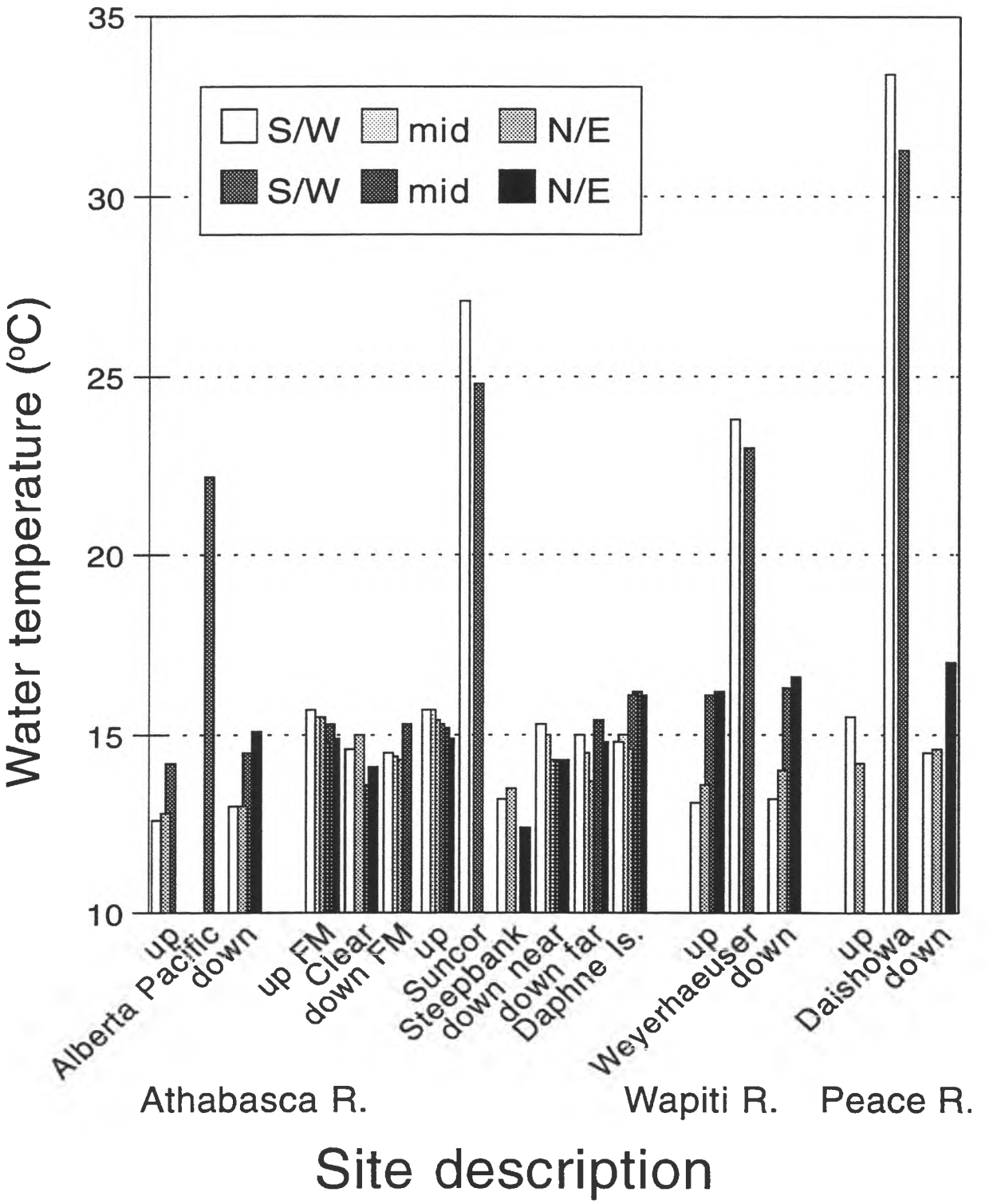

Figure 3. Water temperature at SPMD deployment sites on the Athabasca, Peace and Wapiti Rivers and in final effluent ponds of three pulp mills and wastewater from Suncor, August to September, 1995. Lighter bars represent temperatures on deployment, and darker bars represent temperatures on retrieval of SPMDs.

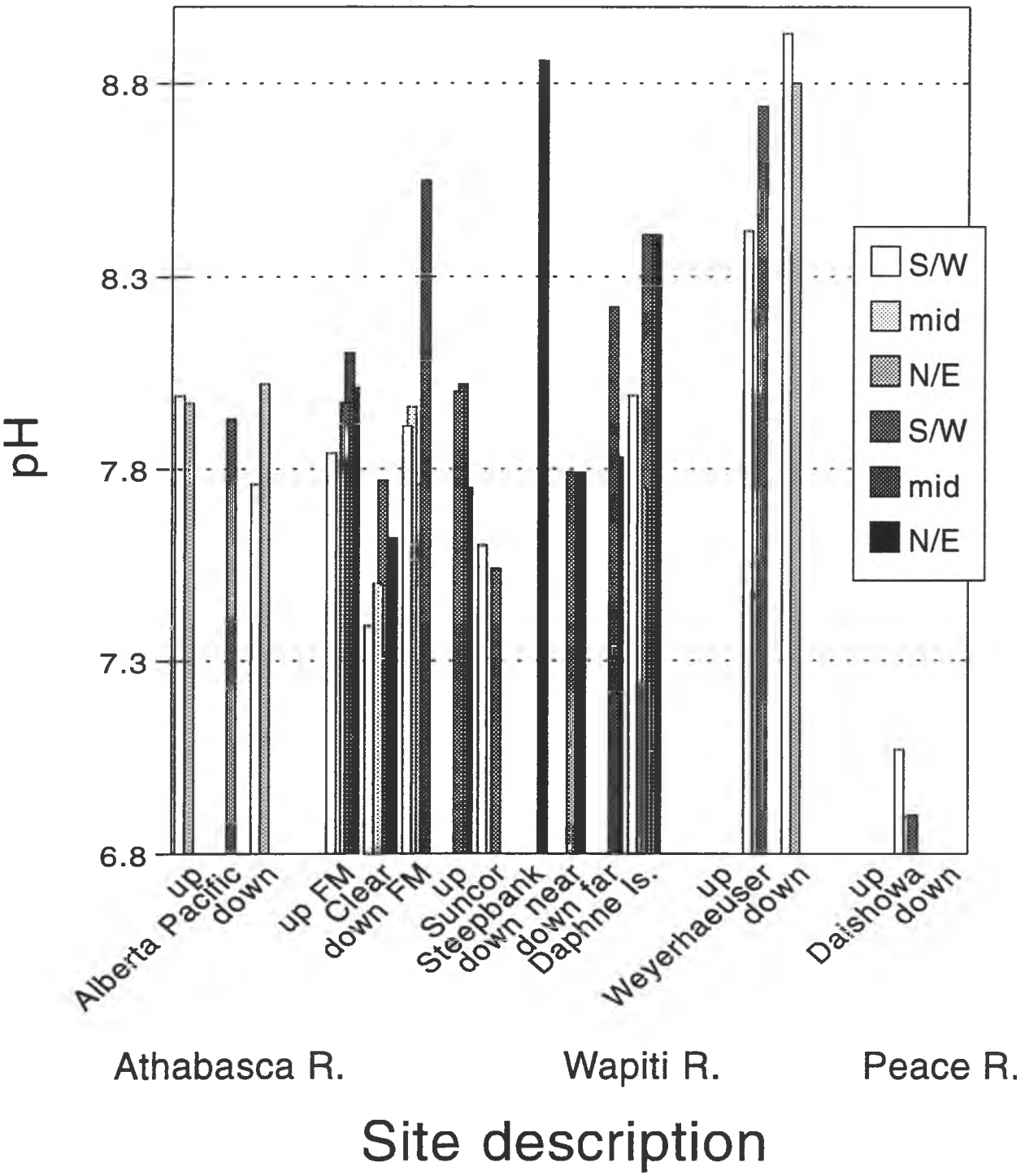

Figure 4. Water pH at SPMD deployment sites on the Athabasca, Peace and Wapiti Rivers and in final effluent ponds of three pulp mills and wastewater from Suncor, August to September, 1995. Lighter bars represent pH on deployment, and darker bars represent pH on retrieval of SPMDs.

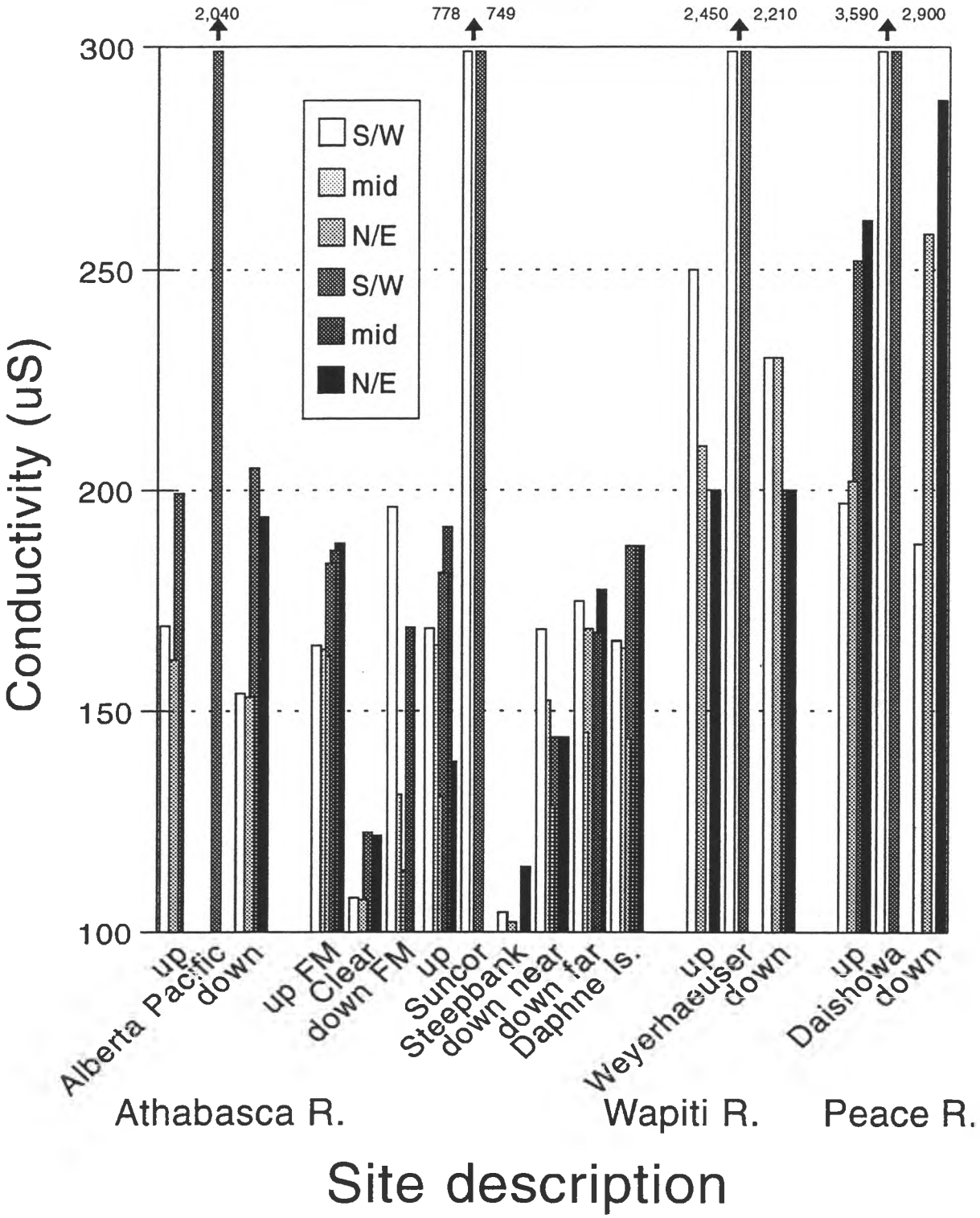

Figure 5. Water conductivity at SPMD deployment sites on the Athabasca, Peace and Wapiti Rivers and in final effluent ponds of three pulp mills and wastewater from Suncor, August to September, 1995. Lighter bars represent conductivity on deployment, and darker bars represent conductivity on retrieval of SPMDs.

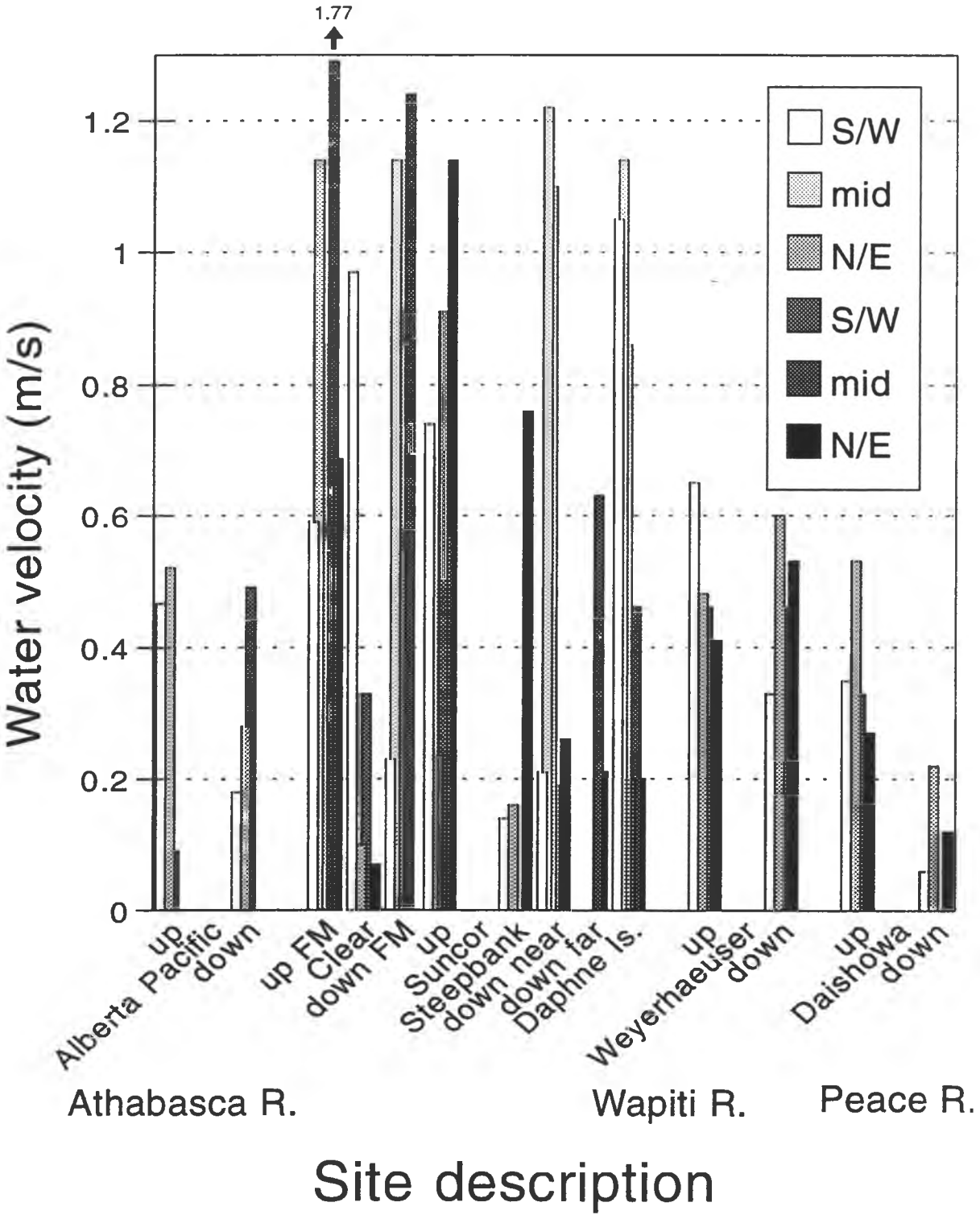

Figure 6. Water velocity (m/sec) at SPMD deployment sites on the Athabasca, Peace and Wapiti Rivers and in final effluent ponds of three pulp mills and wastewater from Suncor, August to September, 1995. Lighter bars represent water velocity on deployment, and darker bars represent water velocity on retrieval of SPMDs.

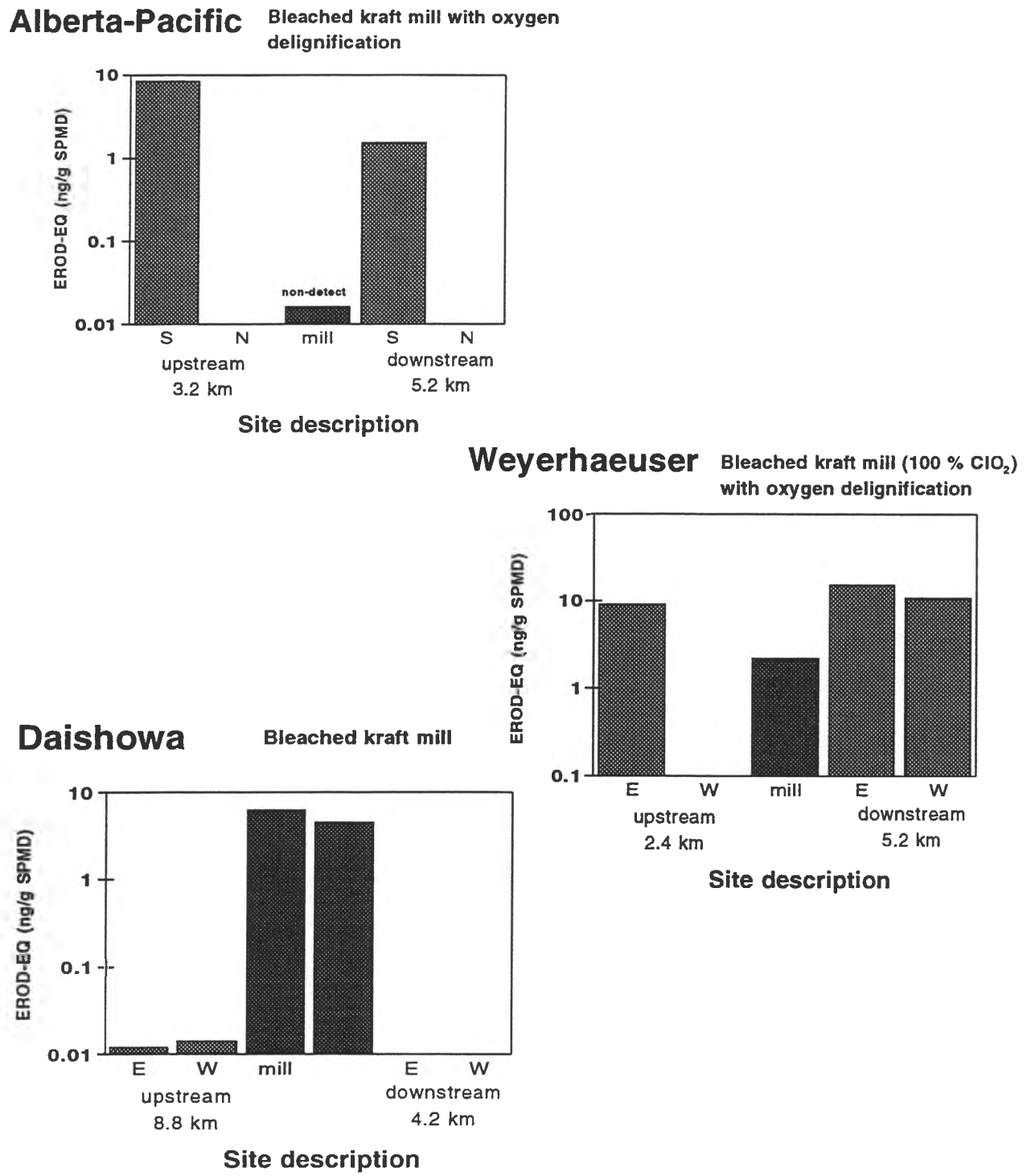

Figure 7a. EROD induction potency (ng EROD-EQ/g SPMD) in fish cells (PLHC-1) exposed to extracts of SPMDs from waters of the Athabasca, Wapiti and Peace Rivers and from three pulp mill effluents.

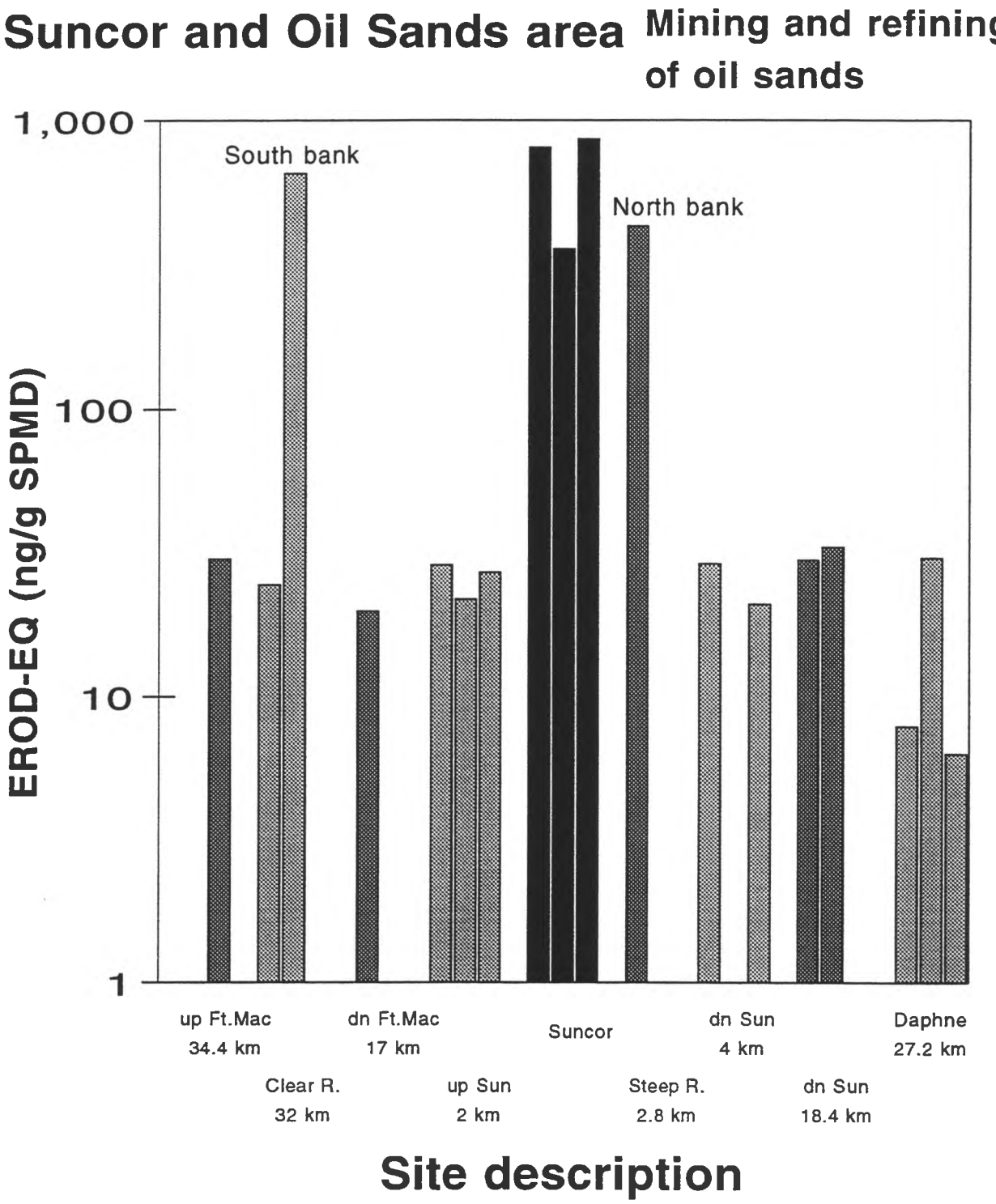

Figure 7b. EROD induction potency (ng EROD-EQ/g SPMD) in fish cells (PLHC-1) exposed to extracts of SPMDs from the Athabasca, Clearwater and Steepbank Rivers in the area of the oil sands and from Suncor refinery wastewater. "Up" refers to upstream sites, and "dn" refers to downstream sites.

Table 3: Numbers of SPMDs deployed and recovered.

Code	Total deployed (devices x SPMDs)	Total recovered (devices x SPMDs)	Comments on lost, removed or exposed deployment devices
Alberta-Pacific Pulp Mill on Athabasca River			
upstream Alberta Pacific	2 x 2	2 x 2	two exposed to air
Alberta Pacific	2 x 2	2 x 2	
downstream Alberta Pacific	2 x 2	2 x 2	one exposed to air
Fort McMurray / Suncor / Oil Sands Area			
upstream Fort McMurray	3 x 2	3 x 2	one exposed to air
Clearwater River	2 x 2	2 x 2	
downstream Fort McMurray	3 x 2	1 x 2	two exposed to air
upstream Suncor	3 x 2	3 x 2	
Suncor	3 x 2	3 x 2	
Steepbank River	2 x 2	2 x 2	one exposed to air
downstream near Suncor	3 x 2	2 x 2	one lost
downstream far Suncor	3 x 2	2 x 2	one removed to shore
Daphne Island	3 x 2	3 x 2	
Weyerhaeuser Pulp Mill on Wapiti River			
upstream Weyerhaeuser	2 x 2	2 x 2	
Weyerhaeuser	2 x 2	2 x 2	
downstream Weyerhaeuser	2 x 2	2 x 2	
Daishowa Pulp Mill on Peace River			
upstream Daishowa	2 x 2	2 x 2	
Daishowa	2 x 2	2 x 2	
downstream Daishowa	2 x 2	1 x 2	one removed to shore
Summary of SPMDs lost:	**one device lost, two devices tampered with (found on shore), seven devices exposed to air = Total 10 devices or 20 SPMDs unusable**		
Grand Total:	**90 deployed SPMDs**	**88 recovered SPMDs**	**70 useable SPMDs**

Table 4. Potencies of SPMD extracts (EC50s and ng EROD-EQ/g SPMD) for induction of EROD activity in PLHC-1 cells. Asterisks indicate triphasic curves where EC50s were estimated by eye.

Station	Side	EC50 %	pg EROD-EQ/g SPMD
upstream Alberta Pacific	south	1.1	8.36
	north		exposed to air
Alberta Pacific	pond	bad d/r[1]	
downstream Alberta Pacific	south	6.9	1.53
	north		exposed to air
upstream Fort McMurray	west	0.42	29.8
	middle	sample missing	
	east		exposed to air
Clearwater River	north	0.39	24.4
	south	0.0021*	655 tri[2]
downstream Fort McMurray	east		exposed to air
	middle	0.5	19.7 tri
	west		exposed to air
upstream Suncor	west	0.4	26.8
	middle	0.46	21.7
	east	0.4	28.4
Suncor	pond	0.017*, 0.024*, 0.020*	807, 358, 860 tri
Steepbank River	north	0.025*	429 tri
	south		exposed to air
downstream Suncor	west	0.46	20.8
	middle		lost in field
	east	0.24	28.6 tri

Station (cont.)	Side	EC50 %	pg EROD-EQ/g SPMD
far downstream Suncor	east	0.42, 0.46	29.5, 23.4 tri
	middle	0.35	32.7
	west		on shore
Daphne Island	east	1.4	7.79
	middle	0.54	22.2 tri
	west	2.5	6.26
upstream Weyerhaeuser	east	1.5	9.06
	west	bad d/r	
Weyerhaeuser	pond	4.6	2.22 bad d/r
downstream Weyerhaeuser	east	bad d/r	
	west	1.4	10.9
upstream Daishowa	west	657	0.014
	east	726	0.012
Daishowa	pond	1.8, 1.6	6.28, 4.50
downstream Daishowa	west		on shore
	east	bad d/r	

* indicates triphasic curves (see Appendix C) where EC50s were estimated by eye from the graph.

[1] "bad d/r" indicates dose response curves that were non-descript and EC50s could not be calculated or estimated with accuracy.

[2] "tri" indicates dose response curves that were triphasic, rising and falling then rising again.

pmol/mg/min (Appendix C, Figure AC.4).

SPMDs from two river sites contained potent EROD inducers: the south side of the Clearwater River (655 ng EROD-EQ/g) and the north side of the Steepbank River (429 ng EROD-EQ/g) (Figure 7b). EC50s were 0.021 to 0.025 % extract dilution, and dose-response maxima were 80 and 40 pmol/mg/min, for Clearwater and Steepbank Rivers, respectively. Both dose-response curves were triphasic (Appendix C, Figure AC.5), so EC50s were estimated by eye from the curves.

SPMDs from the Suncor wastewater were the most potent, with 358 to 860 ng EROD-EQ/g (Figure 7b), although the range for the three replicate samples of 2 SPMDs each overlapped the potencies of the Clearwater and Steepbank sites. Suncor SPMD EC50s were 0.017 to 0.024 % extract dilution, with triphasic curves with maxima of 30 pmol/mg/min (Appendix C, Figure AC.6). EC50s were estimated by eye from the triphasic dose response curves.

3.4 Chemical Analyses of SPMD Extracts

Polyaromatic hydrocarbons (PAHs) in SPMDs

PAH uptake in SPMDs is dependent upon stream velocity, water temperature, time of exposure and the equilibrium partition coefficient between the membrane and compound (Huckins *et al.* 1993). Given the varied sampling conditions, concentrations of PAH (expressed as ng/mL triolein) in dialysate were not used to estimate water concentrations, and therefore not true quantitative measures. Instead, PAH dialysate concentrations were utilized to differentiate between spatial trends. Twenty-eight SPMD dialysates (Appendix D, Table AD.2) were selected and analyzed for thirty-four PAH residues. Concentrations of PAHs in the method blank and six trip blanks (Table AD.3) varied from low (<100 ng/mL) to high (500 to 5000 ng/mL). The highest levels were found in the upstream Alberta Pacific (1B) and Fort McMurray (7B) trip blanks. Both dialysates had greater than anticipated background concentrations, in particular, high concentrations of naphthalene, methyl naphthalene, acenaphthylene and acenaphthene. The pattern of PAH contamination observed in trip blanks was not recognizable in any sample dialysates nor in the SPMD samples associated with these trip blanks. For this reason trip blank concentrations were not referenced when evaluating site-to-site differences. The reproducibility for PAH uptake using SPMD sampling devices was considered good, based on the overall agreement between PAH concentrations in duplicate samples taken at the Weyerhaeuser pond, Daishowa pond and Suncor pond.

Inputs of PAH compounds as a result of pulp and paper operations did not appear to be a significant source. SPMD dialysates from the Alberta Pacific mill ponds I and II (Appendix D, Table AD.4) were only moderately enriched with phenanthrene, methyl phenanthrenes, fluoranthene, pyrene and chrysene compared to upstream and downstream SPMDs. The Weyerhaeuser mill pond had a slight concentration enrichment in phenanthrene and alkyl phenanthrenes (Table AD.5), while the Daishowa mill pond had no discernable difference in PAH patterns or concentrations, relative to upstream SPMD levels (Table AD.6).

Inputs of PAHs from areas within the oil sands region were apparent. SPMDs upstream of Fort

McMurray exhibited low concentrations of PAH compounds. However, downstream of Fort McMurray in the upstream portion of the Clearwater River, fluoranthene, pyrene, alkyl two-ring and alkyl three-ring PAH were observed in SPMDs from the south shore site (Appendix D, Table AD.7). These same type of PAHs were found in the SPMD dialysates from the Steepbank River (*14*) and Daphne Island (*26*) (Table AD.8). Concentrations of PAHs in the Clearwater and Steepbank River dialysates were quite similar, but somewhat lower in the Daphne Island dialysate. Only the Clearwater south (*6*) and Steepbank north (*14*) sites exhibited high MFO activity.

The occurrence of PAHs in SPMDs from the Suncor pond (Appendix D, Table AD.8) were altogether different than the patterns and concentrations observed in river samples. Concentrations of alkyl-PAH were low in comparison to concentrations of the four-ring and five-ring PAH. Concentrations of pyrene in the duplicate Suncor wastewater SPMDs were 40,000 and 23,000 ng/mL, respectively. There was some enrichment of benzo[a]pyrene, benzofluoranthenes and fluoranthene, ranging from 180 to 1300 ng/mL, yet, concentrations of alkyl-phenanthrenes and -anthracenes remained similar to upstream and downstream concentrations.

Organic chemicals in SPMDs

The main chemical constituents (Appendix D, Table AD.9) in SPMD dialysates from pulp and paper mills were nonylphenol, anthraquinone(s), methoxy-hydroxy stilbenes, chlorinated phenolics, chlorinated stilbenes, chlorinated diarylethanes, sulphur and phthalates. The main chemical constituents in SPMD dialysates from sites in the oil sands area were aromatic PAH, alkyl-PAH, naphthenic acids, benzothiophenes and methyl carbazoles.

3.5 EROD induction in fish exposed to Suncor wastewater

In total, 131 fish were exposed to wastewater. For controls, 25 fish were exposed to lab water (negative control fish) and 24 fish were exposed to 10 μg/L BNF (positive control fish). There were no treatment-related deaths in the wastewater exposures. One fish died in 0.1 % effluent, and two BNF-exposed fish died, although this appeared to be related to an aeration failure.

Fish showed dose responsive increases in EROD activity with increasing concentrations of wastewater (Figure 8). Fish exposed to lab water (negative controls) showed low, constitutive levels of EROD activity. Exposure to wastewater caused increases in EROD activity, with a peak activity at 32 % wastewater. Exposure to 10 % wastewater gave EROD activities slightly lower than the positive control fish that were exposed to BNF. Fish exposed to 32 % wastewater for 4 d had EROD activities up to 3 times that of the positive controls. Exposure to 100 % wastewater induced EROD, but activity appeared to be declining (compared to 32 %).

There was no difference in potency between wastewaters sampled on two different dates (September 29 versus October 13), nor was there any difference in potency between wastewater tested immediately and that tested one or two weeks later.

Pooling all results, and using EROD activities for individual fish, a significant ($p<0.001$) regression was

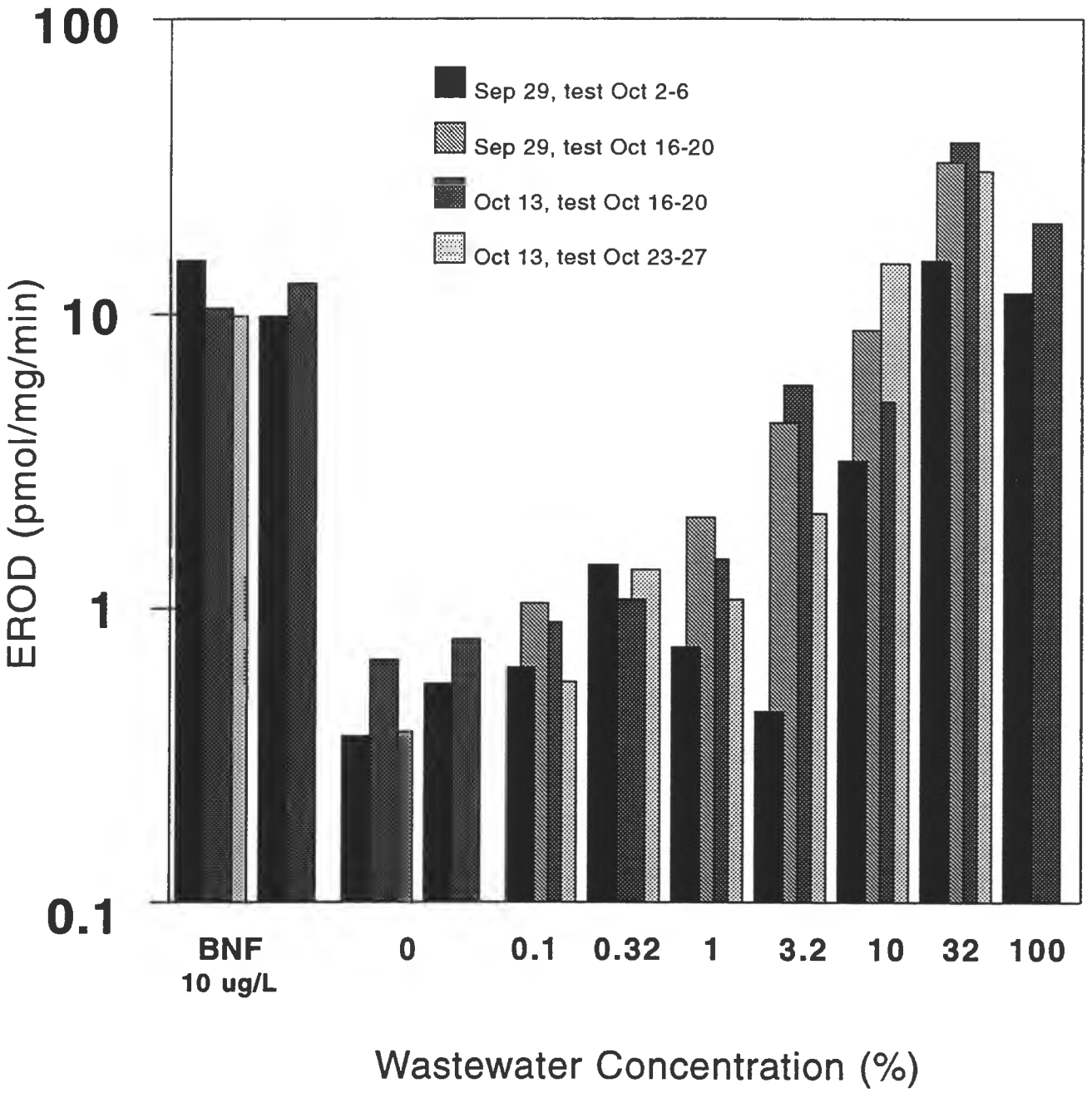

Figure 8. Geometric mean EROD activity in small rainbow trout exposed to Suncor refinery wastewater or B-naphthoflavone for 4 days. Means were calculated from EROD activities of 5 or 6 fish. Darker bars represent tests of the two batches of wastewater when it first arrived, lighter bars represent re-testing of these waters after 1-2 weeks storage.

obtained relating log EROD activity to log wastewater concentration.

$$\log EROD = 0.270 + 0.564 \log CONCN \qquad r^2 = 0.576 \qquad \text{(equation 1)}$$

When mean EROD activities (geometric means of EROD activities of 5 fish in each tank) were used to relate log EROD and log wastewater concentration, a significant ($p<0.001$) regression with a better fit (than equation 1) was obtained, but the lines were very similar.

$$\log EROD = 0.287 + 0.549 \log CONCN \qquad r^2 = 0.750 \qquad \text{(equation 2)}$$

Using Tukey's to compare means of individual fish EROD activities, it was found that the 0.1 % wastewater was not significantly different from the control, the 0.32 and 1.0 % wastewater were just significantly different ($p = 0.040$ to 0.056), and the 3.2 to 100 % wastewater were all significantly different ($p < 0.001$) from control fish EROD activities.

When Tukey's was used for comparison of treatment means (pooling 5 ERODs into a mean for each tank) the model was not as powerful (22 df versus 171 df with individual fish) and so declared fewer treatment differences. Under these conditions, 0.1, 0.32 and 1.0 % wastewater all had similar EROD activities to control fish, and 3.2 to 100 % wastewater were declared different than controls.

EROD activity in control fish was about 0.5 pmol/mg/min (n=25, 95 % CI 0.096-2.5). Using equations 1 and 2, the concentration of wastewater predicted to raise EROD activities just above "threshold" (the upper 95 % CI for control EROD activity) was about 1.6 to 1.7 %. To elevate EROD to the levels of the positive control BNF, about 10 pmol/mg/min, required exposure to about 20 % wastewater.

4.0 DISCUSSION

SPMDs were deployed at 45 sites and successfully retrieved at 44 sites on the Athabasca, Peace and Wapiti Rivers and in effluents of three pulp mills and one oil sands mining and refining effluent. The modified deployment device, with a larger base and heavier gauge aluminum collar than the 1994 device used for deployment, withstood the rough river currents.

Extracts of SPMDs deployed in river waters of the upper Athabasca and the Peace and Wapiti Rivers were low in potency, although the Athabasca River in the oil sands area was significantly higher than in upstream areas. Effluents from the three pulp mills tested in the 1995 survey, Alberta Pacific, Weyerhaeuser and Daishowa, had low potencies of EROD inducers concentrated by SPMDs. This trend was similar to that seen in the 1994 Athabasca survey (Parrott *et al.* 1996) where four Alberta pulp mill effluents, and upper Athabasca and Lesser Slave River waters contained low levels of MFO inducers. Compared to SPMDs deployed in two Ontario bleached kraft mill effluent, the Alberta pulp mill SPMDs contained very low potency MFO inducers (Parrott *et al.* 1994).

Potency of Athabasca river SPMDs increased in the areas of Fort McMurray and the oil sands, a trend

which was also seen in the 1994 sampling. In the 1995 survey, the increased SPMD deployments in the oil sands area detected two hotspots where EROD potencies were high: the south side of the Clearwater River and the north side of the Steepbank River. SPMDs deployed in Suncor wastewater contained high quantities of EROD inducers, similar to the 1994 results.

The absolute numerical potencies of extracts are not comparable between the 1994 and 1995 SPMD surveys, as the methods of calculation of ng EROD-EQ/g differed. In 1994, a slope-ratio assay was used for determining potency, while in 1995 we used a comparison of EC50s from the dose response curves. Although numerical comparisons cannot be made, the trends seen in the 1994 survey were repeated in 1995. Low induction was seen in pulp mill effluents and in background waters upstream of the oil sands. Oil sands area SPMDs were higher in potency and were more variable. Suncor SPMDs contained potent MFO inducers. In the 1995 survey, two tributaries of the Athabasca River in the oil sands area were sampled, the Steepbank and the Clearwater Rivers. These proved to be high in MFO inducing chemicals.

The induction of fish cells exposed to SPMD extracts from Suncor wastewaters was mirrored by the induction in small fish exposed in the lab to the wastewaters. Exposure of small rainbow trout to Suncor wastewater for 4 d induced hepatic EROD activity. Induction was the greatest at 32 % effluent, and was up to three times higher than EROD activity in fish exposed to positive control BNF. Induction at 100 % was slightly less than at 32 %, suggesting possible inhibition or toxicity of the wastewater.

Regressions comparing log EROD activity to log wastewater exposure concentration showed that the threshold for induction of EROD significantly above control activities was 1.6 to 1.7 % wastewater. To elevate EROD to the level of the positive control, BNF, required exposure to about 20 % wastewater. This concentration of effluent would not be found in the Athabasca River, where dilution results in about 1 % effluent.

Both small fish and fish cells responded to the Suncor wastewater with EROD induction. Fish cells did not achieve very high induction maxima (only one-tenth of the TCDD induction maxima), similar to the live fish, which were induced as high as the positive control (BNF) but not as high as trout exposed to TCDD (500 pmol/mg/min, Parrott *et al.* 1995a). Fish cells responded to extremely small amounts (0.02 %) of the SPMD extract, so their sensitivity was high.

The detection of two induction hotspots in the oil sands area on tributaries of the Athabasca River (the Clearwater and Steepbank Rivers) suggests natural erosion of the oil sands contributes potent MFO inducers in these areas. The Clearwater River SPMDs detected a localized source of inducers, as SPMDs deployed on the south side were high while SPMDs on the north side were similar to the background induction potency for Athabasca River waters in the oil sands area. It is unknown whether the high potency of SPMDs from the north side of the Steepbank River represents a localized plume or erosional source, as SPMDs from the south side were lost (vandalized).

The dose response curves induction of EROD in PLHC-1 were peculiar. In the oil sands area, a common trend was a triphasic type of dose response, where induction in the fish cells increased and decreased, then increased again. Rather than some error in the dilution or dosing protocol, these triphasic curves

appear to be real, as composite samples of separate SPMDs deployed in Suncor wastewater all showed the same triphasic pattern. This, together with the observation of the triphasic dose responses only in the oil sands area, suggests a real effect of the inducing chemical(s) from the oil sands area.

Determining potency from the triphasic curves was difficult, and potencies were calculated by eye in some cases. This pattern of response could indicate two (or more) inducers were present, one acting at low concentrations and one at high. Another possibility is that solubility of the inducers affected the response, and the triphasic curves were caused by inducers that were at various stages of solubility/insolubility as the extracts were diluted for dosing the cells. Another possibility is suppression of the EROD activity by the inducer or by some other chemical(s) in the complex SPMD extract. EROD activity has been shown to be suppressed by some inducers present in the cell exposure media. Hahn *et al.* (1996) reported low EROD activity in PLHC-1 cells exposed to PCBs, while the CYP1A protein was maximally induced. They suggested inhibition or inactivation of catalytic function may occur at high concentrations of the inducer. We would not detect suppression of EROD activity in the present samples, as CYP1A protein was not determined. If activity was suppressed, and induction continued past the point where the triphasic curves decrease, then the potencies of SPMD extracts would be overestimated. Although we do not know whether suppression is occurring, if it is, the responses would be overestimated for several oil sands sites, for the Suncor wastewater as well as for the hotspot areas of the Clearwater and Steepbank Rivers.

SPMD sampling rates, membrane fouling and water velocity effects on uptake of inducers have been discussed previously (Parrott *et al.* 1996). The effects of temperature on SPMD uptake of MFO inducting chemicals has also been described, but should be discussed in relation to the Clearwater and Steepbank Rivers SPMDs. Temperature has been shown to affect the uptake of compounds into SPMDs, but for rigidly-structured PAH compounds, the effects of temperature are minimal (Huckins *et al.* 1995). Nevertheless, the fact that SPMDs accumulated high concentrations of MFO inducers from the 25 to 27 °C Suncor wastewater and from the 12 to 15 °C waters of the Clearwater and Steepbank Rivers is noteworthy. If there was a modest increase in uptake in the Suncor SPMDs over the 12 °C temperature gradient, then Clearwater and Steepbank SPMDs can be viewed as equal in potency to Suncor wastewater exposed SPMDs.

It is not known which chemicals in the SPMD extracts were responsible for the observed MFO induction in fish cells. Classical MFO inducers are chlorinated dibenzo-p-dioxins, furans and PCBs, as well as certain PAHs such as benzo(a)pyrene (BAP). SPMDs from the Clearwater River and Suncor wastewater contained BAP, but those from the Steepbank River did not. So while BAP may have contributed to the induction seen in Suncor and Clearwater SPMDs, it could not have caused significant induction at other sites.

The three extracts highest in potency, the Suncor wastewaters, the south Clearwater River and the north Steepbank River, all contained high concentrations of PAHs. Patterns of PAHs within these MFO-inducing SPMDs differed. River site-SPMDs showed high concentrations of acenaphthylene, acenaphthene, fluorene, phenanthrene and methyl and dimethyl phenanthrene/anthracenes, compared to Suncor-SPMDs. Conversely, Suncor wastewater SPMD extracts contained higher concentrations of

pyrene than potent MFO-inducing SPMDs from Steepbank or Clearwater Rivers.

Di-, tri- and tetra-methyl phenanthrenes have been shown to induce MFO (Parrott *et al.* 1995b). Fish exposed in the lab to 3,6-dimethyl phenanthrene show MFO induction, so the dimethyl phenanthrenes in the Clearwater and Steepbank SPMDs may possibility be inducing MFO. However, concentrations of dimethyl phenanthrenes in Suncor extracts are lower, so these chemicals may not be contributing to the induction seen in all extracts. Trimethyl naphthalenes, phenanthrenes and anthracenes were not measured in the PAH analyses, but some of these compounds have been shown to induce MFO in fish in the lab (Parrott *et al.* 1995b).

Examining the list of PAHs, it is clear that several possible MFO inducers were present in the SPMD extracts, and that the compounds concentrated by the SPMDs appear to differ between oil sands area river sites and Suncor wastewater. The chemicals measured represent only a few of the hundreds of compounds present in the complex mixture concentrated by the SPMDs, so we are unable to discern the MFO inducer(s). It is clear that there are many "candidate" compounds, even from the limited list we did quantify in the MFO-inducing SPMD extracts.

5.0 CONCLUSIONS

SPMDs deployed at most sites on the Athabasca, Peace and Wapiti Rivers accumulated few MFO inducers (<10 ng EROD-EQ/g SPMD). Background concentrations in the Athabasca River in the area of the oil sands were higher (up to 30 ng EROD-EQ/g SPMD). Very high levels of inducers were detected in two Athabasca River tributaries, the Clearwater and Steepbank Rivers. Similar to the 1994 results, low levels of inducers were detected in the final effluents from three pulp mills, Alberta Pacific, Weyerhaeuser and Daishowa. Also similar to the 1994 SPMD survey, potent inducers were detected in Suncor wastewater, though the range overlapped with the Clearwater and Steepbank River sites. Many PAHs were detected in the Suncor, Clearwater and Steepbank SPMDs, and the pattern of contaminants was different for Suncor compared to the river sites: Suncor wastewater SPMD extracts contained higher concentrations of pyrene than SPMDs from the Steepbank or Clearwater Rivers. Fish exposed to Suncor wastewater showed strong induction, similar to PLHC-1 cells exposed to the Suncor SPMD extracts. Triphasic dose response curves seen in PLHC-1 cells doses with SPMD extracts from the oil sands area require more investigation.

In general, most SPMD extracts were of low potency, with the exception of SPMDs from Suncor wastewater and from the Clearwater and Steepbank Rivers. This suggests inducers released in the high temperature processing and refining of the oil sands may also be released naturally by erosion or weathering of the oil sands. Furthermore, the highly variable induction observed in the oil sands area suggests that release of the inducers into the water is dependent on local weathering/erosion conditions.

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NORTHERN RIVER BASINS STUDY

APPENDIX A - TERMS OF REFERENCE

Project 2354-E1: Semi-Permeable Membrane Devices - Athabasca, Peace and Wapiti Rivers

I. Background and Objectives

Mixed function oxygenases (MFOs) are a family of membrane-bound, detoxification enzymes found in the liver which increase the water solubility of aromatic and lipophilic compounds. Some MFOs break down endogenous compounds such as steroid hormones and fatty acids, and others aid in drug metabolism and the breakdown and excretion of other exogenous compounds. MFO activity is "induced" by the presence of several foreign compounds ; i.e., in the presence of these foreign compounds, animals synthesize new amounts of P-450IA proteins and enzyme activity is measurably increased.

The ability of a compound to induce MFO activity appears to be related to molecular shape, i.e., the co-planarity of connected aromatic rings and the distribution of substituents such as chlorine atoms. Experimental treatments with pure compounds have established that some polycyclic aromatic hydrocarbons and some chlorinated aromatic hydrocarbons induce liver MFO activity in several fish species. Complex mixtures such as Aroclors, petroleum oils and pulp mill effluents also have inducing properties, probably because these mixtures contain specific inducers.

The biological significance of liver MFO induction is not completely known. Induction is an adaptive response and can result in the metabolism and excretion of exogenous substrates. Studies demonstrating increases in MFO activity in fish, birds and mammals have also documented changes in performance, including altered steroid hormone profiles, changes in thyroxine and vitamin A metabolism, impairment of reproductive and immune system, and an increased prevalence of diseases. However, there has not yet been a demonstration of causal links between altered MFO activity and all of the other biochemical responses.

The simultaneous occurrence of adverse effects and MFO induction indicates that measures of MFO induction justify further studies of biological impacts. MFO induction is one of the easiest and most sensitive responses to detect and has therefore been adopted in a wide range of environmental monitoring programs. Measurement of liver MFO induction signals only an increased probability of a suite of associated responses.

As an alternative to collecting live fish, MFO-inducing compounds can be sampled using Semi-Permeable Membrane Devices (SPMDs). SPMDs are polyethylene dialysis bags filled with triolein, a purified fish lipid. This device is a passive *in-situ* sampler that when immersed in water, absorbs fat soluble chemicals with a molecular weight of about 600 or less. These compounds diffuse through pores in the membrane and dissolve in the lipid. The SPMDs

accumulate lipophilic compounds in a very similar way to live fish and to equivalent levels, allowing them to be used as surrogate fish (Huckins et al. 1994). The compounds are recovered either by direct removal of the lipid or by solvent dialysis. SPMDs provide a sampling technique that allows traditional chemical analyses and bioassays to estimate levels of compounds with specific bioactivity (eg., inducers of MFOs).

A previous NRBS project (2354-D1) deployed SPMDs for a 2 week period in the Athabasca River in August and September, 1994, both upstream and downstream of pulp mill and refinery effluents. Extracts of SPMDs from pulp mills were two to five times as potent (ranging from 29.7-62.0 pg TCDD-EQ/g, expressed as the comparable toxic potency of 2,3,7,8-tetrachlordibenzo-*p*-dioxin) as extracts form SPMDs exposed to background river water (Parrott et al. 1995). The concentrations of MFO inducers in SPMDs exposed to river water increased downstream of Fort McMurray. In this area, accumulated inducers in SPMD extracts were highly variable and ranged from 58.5 to 728 pg TCDD-EQ/g, suggesting an unknown source that may be from natural erosion of the tar sands. SPMDs deployed in effluent from Suncor accumulated the most MFO-inducing compounds (16,800 pg TCDD-EQ/g), with concentrations >1000x background river water and >20x the concentrations accumulated in river water upstream of Suncor.

Results from the 1994 study suggest that the four pulp mill effluents sampled on the Athabasca River contribute small quantities of MFO inducing compounds to the Athabasca River. By contrast, very high quantities of MFO inducers were accumulated from the Suncor effluent, in addition to areas immediately upstream near Fort McMurray. The project proposed for 1995 has two major objectives: (1) Further investigate Suncor effluent and surrounding sites on the Athabasca River, and test MFO induction in fish cell lines exposed to extracts of SPMDs versus MFO induction in live fish held on site; (2) Deploy SPMDs at three previously untested pulp mills to compare the results and expand the survey.

II. General Requirements

This project will involve the deployment of the SPMDs at sites along the Athabasca River, Wapiti River and Peace River between August 1 and August 31, 1995. Ideally, the sampling should occur during a declining hydrograph and at temperatures between 15 and 20°C. There two parts to this study.

MFO Inducers in the Effluent at Suncor

This portion of the project is designed to conduct additional tests for MFO inducing compounds in the Suncor effluent and compare MFO induction from SPMD extracts in fish cell lines to MFO induction in live fish. The field work will involve redeployment of SPMDs upstream and downstream of Suncor, and in the refinery effluent itself. In addition, effluent will be shipped to Golder Associates Ltd. laboratory to perform fish and SPMD exposures under control conditions. Cultured rainbow trout will be used in the laboratory investigations. Subsequent laboratory analyses will measure MFO induction in cultured fish cell lines exposed to SPMD extracts and

compare with MFO induction from the laboratory fish. The contractor will also perform traditional chemical analyses of fish tissues and SPMD extracts to determine concentrations and identities of accumulated chemicals.

The contractor is also required to collect bottom sediments from SPMD sites and test for MFO induction in fish in the labs at NWRI, to determine contributions from historical contamination and natural oil seeps.

Expand Field Deployment of SPMDs

The objective of this portion of the project is to replicate and expand on the work done in 1994. SPMDs will be deployed at two formerly untested mills on the Wapiti and Peace rivers; Weyerhaeuser on the Wapiti River and Daishowa on the Peace River. The contractor will also redeploy SPMDs at Alberta-Pacific on the Athabasca River to replace the samplers that were lost last summer.

Details of Sampling Using SPMDs

A. SPMDs are to be prepared and deployed in duplicate to sample compounds that cause MFO induction in the effluent from each of the effluent sources and the receiving water upstream of the mixing zone (reference) of each of the effluents. Downstream from each effluent source where no other industries or towns discharge waste, six SPMDs will be deployed to increase sample volumes and the ability to detect inducers. Specifically, the SPMDs are to be deployed at the following locations:

i) upstream of Alpac - Athabasca River (2);
ii) in the Alpac effluent discharge (2);
iii) downstream of Alpac (6);
iv) upstream of Weyerhaeuser - Wapiti River (2)
v) in the Weyerhaeuser effluent discharge (2)
vi) downstream of Weyerhaeuser (6)
vii) upstream of Daishowa - Peace River (2)
viii) in the Daishowa effluent discharge (2)
ix) downstream of Daishowa (6)
x) 2 sites upstream of Suncor - Athabasca River (4)
xi) in the Suncor effluent discharge (3 reps) (6)
xii) 2 sites downstream of the tar sands (4)

( ) indicates the number of SPMDs to be deployed at each site.

A total of 12 blank procedural field controls are to be included as part of the sampling program. They will be submitted to the laboratories as a Quality Assurance/Quality Control measure of sampling and analytical methods.

B. The latitude and longitude of each sampling location is to be recorded in the field using Geographic Positioning Technology.

C. SPMDs will be left in the river for a period of two weeks. Given the vast dilution factor in the Peace and Athabasca rivers, two weeks should allow sufficient time for the diffusion and accumulation of fat soluble chemicals through the membranes.

D. Flow rates (using a hand-held flow meter) and water temperatures will be recorded at each sampling site during the sampling period. Discharge rates will be recorded subsequently by accessing National Hydrologic Survey data.

E. After two weeks the SPMDs will be taken from the water, packaged in sealed containers, placed in coolers and frozen for shipment. All samples must be maintained at -20°C during shipping.

F. Following their retrieval, the SPMDs will be returned to the lab, extracted into solvent and split for analysis (see Huckins et al. 1994). The samples will be tested for MFO inducing ability with cell culture techniques using fish, bird and mammalian cell line assays. Those samples showing MFO induction will undergo traditional chemical analyses for the presence of suspect compounds.

G. The presence of MFO-inducers in SPMD extracts will be assayed using fish cell cultures. **Potency (i.e., amount of inducer) will be expressed as picograms of 2,3,7,8 - TCDD equivalents based on cell lines exposed to standard levels of dioxins.** These data will be compiled and comparisons of induction made to mill characteristics and the distance from the effluent discharges.

III. Reporting Requirements

1. A brief progress report is to be sent to the Study Office by **September 30, 1995**. Ten copies of the Draft Report along with an electronic disk copy are to be submitted to the Component Coordinator by **October 31, 1995**.

2. Data / Sample Deliverables:
   - MFO analysis data on individual fish (rainbow trout), 3 weeks after the end of exposure,

- MFO analysis data on fish cells exposed to SPMD extracts from all concentrations in the Suncor wastewater (pond water) exposure experiments, 8 weeks after termination of the exposures,

- MFO analysis data on fish cells exposed to SPMD extracts deployed in the Fort McMurray area and around Suncor, 8 weeks after termination of the exposures,

- flow, pH and temperature data from all SPMD deployment sites in the Fort McMurray /Suncor area, one week after the collection of the SPMDs,

- MFO analyses of laboratory fish and MFO analyses of fish cells exposed to SPMD extracts from deployments off the Steepbank River and a reference site, 8 weeks after termination of the exposures,

- MFO analyses of laboratory fish exposed to sediments or sediment extracts from selected SPMD deployment sites, 10 weeks after the collection of sediments,

- Extracts of SPMDs (dialysates) from the Suncor effluent and from deployments in the Fort McMurray / Suncor area to be archived for future analysis, 4 weeks after withdrawal of SPMDs from the river,

3. **Three weeks after the receipt of review comments on the draft report**, the Contractor is to provide the Component Coordinator with two unbound, camera ready copies and ten cerlox bound copies of the final report along with an electronic version.

4. The Contractor is to provide draft and final reports in the style and format outlined in the NRBS document, **"A Guide for the Preparation of Reports,"** which will be supplied upon execution of the contract.

   The final report is to include the following: an acknowledgement section that indicates any local involvement in the project, Report Summary, Table of Contents, List of Tables, List of Figures and an Appendix with the Terms of Reference for this project (include an addendum to explain any variances from the original TOR).

   Text for the report should be set up in the following format:

   a) Times Roman 12 point (Pro) or Times New Roman (WPWIN60) font.
   b) Margins; are 1" at top and bottom, 7/8" on left and right.
   c) Headings; in the report body are labelled with hierarchical decimal Arabic numbers.
   d) Text; is presented with full justification; that is, the text aligns on both left and right margins.

e) Page numbers; are Arabic numerals for the body of the report, centred at the bottom of each page and bold.

- If photographs are to be included in the report text they should be high contrast black and white.
- All tables and figures in the report should be clearly reproducible by a black and white photocopier.
- Along with copies of the final report, the Contractor is to supply an electronic version of the report in Word Perfect 5.1 or Word Perfect for Windows Version 6.0 format.
- Electronic copies of tables, figures and data appendices in the report are also to be submitted to the Component Coordinator along with the final report. These should be submitted in a spreadsheet (Quattro Pro preferred, but also Excel or Lotus) or database (dBase IV) format. Where appropriate, data in tables, figures and appendices should be geo-referenced.

5. All figures and maps are to be delivered in both hard copy (paper) and digital formats. Acceptable formats include: Harvard Graphics 3.0, DXF, uncompressed Eøø, VEC/VEH, Atlas and ISIF. All digital maps must be properly geo-referenced.

6. All sampling locations presented in report and electronic format should be geo-referenced. This is to include decimal latitudes and longitudes (to six decimal places) and UTM coordinates. The first field for decimal latitudes / longitudes should be latitudes (10 spaces wide). The second field should be longitude (11 spaces wide).

7. A presentation package of 35 mm slides is to comprise of one original and four duplicates of each slide.

IV. Deliverables

Following analyses of the data, a **Draft Report** is to be submitted by **October 31, 1995**, along with ten to twenty 35 mm slides that can be used at public meetings to summarize the project, methods and key findings.

V. Contract Administration

This project has been proposed by the Contaminants Component of the NRBS (Contaminants Component Leader - Dr. John Carey, NWRI, Burlington).

The Scientific Authority for this project is:

Dr. Joanne Parrott
National Water Research Institute
867 Lakeshore Road
P.O. Box 5050
Burlington, Ontario
L7R 4A6
phone: (905) 336-4551
fax: (905) 336-6430

Questions of a technical nature should be directed to her.

The NRBS Study Office Component Coordinator for this project is:

Richard Chabaylo
Office of the Science Director
Northern River Basins Study
690 Standard Life Centre
10405 Jasper Avenue
Edmonton, Alberta
T5J 3N4
phone: (403) 427-1742
fax: (403) 422-3055

Administrative questions related to this project should be directed to him.

VI. Literature Cited

Huckins, J. H., J. D. Petty. J. A. Lebo and J. L. Zajicek. 1994. SPMD Technology. Tutorial. U.S. Dept. of Interior, National Biological Survey, Columbia Missouri. 62 pp.

Parrott, J.L., P.V. Hodson, D.E. Tillitt, D.T. Bennie and M.E. Comba. 1995. Semipermeable membrane devices (SPMDs) accumulate inducers of fish fixed function oxygenase (MFO) from four pulp mills and one refinery on the Athabasca River system, Alberta, Canada. Draft report prepared for Northern River Basins Study, Edmonton, Alberta. Project No. 2354-D1.

Canada Alberta Northwest Territories

Northern River Basins Study

FACSIMILE COVER SHEET

FEEDBACK REQUIRED

ATTENTION: JOANNE PARROTT, Ph.D
RESEARCHER
NATIONAL WATER RESEARCH INSTITUTE
ENVIRONMENT CANADA
P.O. BOX 5050
BURLINTON, ONTARIO L7R 4A6

FAX NO: 905 - 336 - 6430

PHONE NO: 905 - 336 - 4551

FROM: KEN CRUTCHFIELD
ASSOCIATE SCIENCE DIRECTOR

June 27, 1995

NO. OF PAGES INCLUDING COVER SHEET: 4
(ORIGINAL NOT TO FOLLOW)

OUR FILE: 2354 - E1

SUBJECT: *REVISIONS* TO SPMD TOR

Attached are the pages modified to fit the changes noted in your June 20th facsimile. The data deliverables have been added as reporting deliverables to facilitate Suncor in preparation of their contract with NWRI. Suncor will likely reference the NRBS contract and specifically this section. They will receive the data from NRBS. Unless otherwise advised by the Board the following process will be followed once the *Component Leader* indicates the data is scientifically accepted. Upon reciept of this confirmation the Study Board has 30 days to file an objection to public release, beyond which the data is in the public domain.

KEN

Attachment (3pg)
c.c F. Wrona - attm't - FAX
W. Gummer - attm't - FAX

#690 Standard Life Centre, 10405 Jasper Avenue, Edmonton, Alberta, T5J 3N4
(403)427-1742 FAX(403)-422-3055

MFO Inducers in the Effluent at Suncor

This portion of the project is designed to conduct additional tests for MFO inducing compounds in the Suncor effluent and compare MFO induction from SPMD extracts in fish cell lines to MFO induction in live fish. The field work will involve redeployment of SPMDs upstream and downstream of Suncor, and in the refinery effluent itself. In addition, **effluent will be shipped to Golder Associates Ltd. laboratory to perform fish and SPMD exposures under control conditions**. Cultured rainbow trout will be used in the laboratory investigations. Subsequent laboratory analyses will measure MFO induction in cultured fish cell lines exposed to SPMD extracts and compare with MFO induction from the **laboratory** fish. The contractor will also perform traditional chemical analyses of fish tissues and SPMD extracts to determine concentrations and identities of accumulated chemicals.

The contractor is also required to collect bottom sediments from SPMD sites and test for MFO induction in fish in the labs at NWRI, to determine contributions from historical contamination and natural oil seeps.

Expand Field Deployment of SPMDs

The objective of this portion of the project is to replicate and expand on the work done in 1994. SPMDs will be deployed at two formerly untested mills on the Wapiti and Peace rivers; Weyerhaeuser on the Wapiti River and Daishowa on the Peace River. The contractor will also redeploy SPMDs at Alberta-Pacific on the Athabasca River to replace the samplers that were lost last summer.

Details of Sampling Using SPMDs

A. SPMDs are to be prepared and deployed in duplicate to sample compounds that cause MFO induction in the effluent from each of the effluent sources and the receiving water upstream of the mixing zone (reference) of each of the effluents. Downstream from each effluent source where no other industries or towns discharge waste, six SPMDs will be deployed to increase sample volumes and the ability to detect inducers. Specifically, the SPMDs are to be deployed at the following locations:

i) upstream of Alpac - Athabasca River (2);
ii) in the Alpac effluent discharge (2);
iii) downstream of Alpac (6);
iv) upstream of Weyerhaeuser - Wapiti River (2)
v) in the Weyerhaeuser effluent discharge (2)
vi) downstream of Weyerhaeuser (6)

III. Reporting Requirements

1. A brief progress report is to be sent to the Study Office by **September 30, 1995.** Ten copies of the Draft Report along with an electronic disk copy are to be submitted to the Component Coordinator by **October 31, 1995.**

2. **Data / Sample Deliverables:**

- MFO analysis data on individual fish (rainbow trout), 3 weeks after the end of exposure,

-MFO analysis data on fish cells exposed to SPMD extracts from all concentrations in the Suncor wastewater (pond water) exposure experiments, 8 weeks after termination of the exposures,

-MFO analysis data on fish cells exposed to SPMD extracts deployed in the Fort McMurray area and around Suncor, 8 weeks after termination of the exposures,

- flow, pH and temperature data from all SPMD deployment sites in the Fort McMurray /Suncor area, one week after the collection of the SPMDs,

- MFO analyses of laboratory fish and MFO analyses of fish cells exposed to SPMD extracts from deployments off the Steepbank River and a reference site, 8 weeks after termination of the exposures,

- MFO analyses of laboratory fish exposed to sediments or sediment extracts from selected SPMD deployment sites, 10 weeks after the collection of sediments,

- Extracts of SPMDs (dialysates) from the Suncor effluent and from deployments in the Fort McMurray / Suncor area to be archived for future analysis, 4 weeks after withdrawal of SPMDs from the river,

2. **Three weeks after the receipt of review comments on the draft report,** the Contractor is to provide the Component Coordinator with two unbound, camera ready copies and ten cerlox bound copies of the final report along with an electronic version.

3. The Contractor is to provide draft and final reports in the style and format outlined in the NRBS document, **"A Guide for the Preparation of Reports,"** which will be supplied upon

IV. Deliverables

Following analyses of the data, a **Draft Report** is to be submitted by **October 31, 1995**, along with ten to twenty 35 mm slides that can be used at public meetings to summarize the project, methods and key findings.

V. Contract Administration

This project has been proposed by the Contaminants Component of the NRBS (Contaminants Component Leader - Dr. John Carey, NWRI, Burlington).

The Scientific Authority for this project is:

Dr. Joanne Parrott
National Water Research Institute
867 Lakeshore Road
P.O. Box 5050
Burlington, Ontario — phone: (905) 336-4551
L7R 4A6 — fax: (905) 336-6430

Questions of a technical nature should be directed to her.

The NRBS Study Office Component Coordinator for this project is:

Richard Chabaylo
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Northern River Basins Study
690 Standard Life Centre
10405 Jasper Avenue
Edmonton, Alberta — phone: (403) 427-1742
T5J 3N4 — fax: (403) 422-3055

Administrative questions related to this project should be directed to him.

APPENDIX B Water Quality

Table AB.1: Temperature, pH, conductivity and water velocity at SPMD deployment sites.

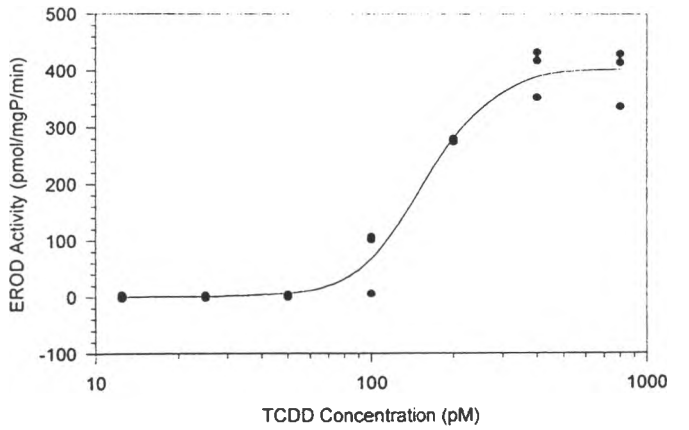
Station	Side	Date	in / out	Veloc. (m/s)	Temp. (C)	Cond. (uS)	pH
upstream Alberta Pacific	south	Aug 13	in	0.466	12.6	169.4	7.99
		Aug 27	out	0.09	14.2	199.3	n/a
	north	Aug 13	in	0.52	12.8	161.7	7.97
		Aug 27	out				
Alberta Pacific	pond	Aug 14	in	mill data			
		Aug 28	out		22.2	2.04 mS	7.93
downstream Alberta Pacific	south	Aug 13	in	0.18	13	154	7.76
		Aug 27	out	0.49	14.5	205	n/a
	north	Aug 13	in	0.28	13	153.1	8.02
		Aug 27	out	n/a	15.1	194	n/a
upstream Fort McMurray	west	Aug 16	in	0.59	15.7	165	7.84
		Aug 30	out	0.65	14.8	183.5	7.97
	east	Aug 16	in	0.86	15.5	162.5	7.81
		Aug 30	out	0.687	14.9	188.1	8.01
	middle	Aug 16	in	1.14	15.5	164.1	7.82
		Aug 30	out	1.77	15.3	186.4	8.1
Clearwater River	north	Aug 16	in	0.1	15	107.5	7.5
		Aug 30	out	0.07	14.1	122.1	7.62
	south	Aug 16	in	0.97	14.6	108	7.39
		Aug 30	out	0.33	13.6	122.7	7.77

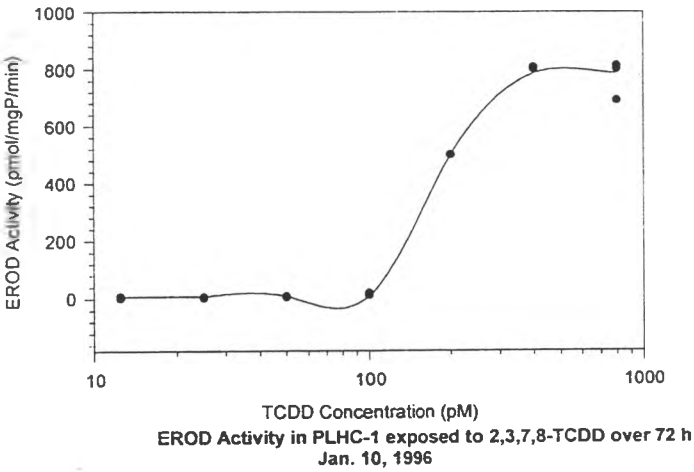
Station	Side	Date	in / out	Veloc. (m/s)	Temp. (C)	Cond. (uS)	pH
downstream Fort McMurray	east	Aug 20	in	1.14	14.3	114.2	7.76
		Sept 03	out				
	west	Aug 20	in	0.23	14.5	196.2	7.91
		Sept 02	out				
	middle	Aug 20	in	1.14	14.4	131.2	7.96
		Sept 02	out	1.24	15.3	169.1	8.55
upstream Suncor	west	Aug 18	in	0.74	15.7	168.8	
		Sept 01	out	0.91	15.3	181.4	8
	east	Aug 18	in	0.24	15.4	130.8	(9.05?) err
		Sept 01	out	1.14	14.9	138.6	7.75
	middle	Aug 18	in	0.687	15.7	165	(9.27?) err
		Sept 01	out	0.57	15.2	191.7	8.02
Suncor	pond	Aug 17	in		27.1	778	7.6
		Aug 31	out		24.8	749	7.54
Steepbank River	north	Aug 18	in	0.16	13.5	102.4	(12.9?) err
		Sept 01	out	0.76	12.4	115	8.86
	south	Aug 18	in	0.14	13.2	104.6	n/a
		Sept 01	out				
downstream Suncor	west	Aug 19	in	0.21	15.3	168.6	(11.3?) err
		Sept 02	out	0.19	14.3	144.1	7.79
	east	Aug 19	in	1.1	14.3	128.8	(12.12?) err
		Sept 02	out	0.26	14.3	144.1	7.79
	middle	Aug 19	in	1.22	15	152.3	n/a
		Sept 02	out				

Station	Side	Date	in / out	Veloc. (m/s)	Temp. (C)	Cond. (uS)	pH
far downstream Suncor	east	Aug 19	in	n/a	13.7	168.7	n/a
		Sept 02	out	0.21	14.8	177.6	7.83
	west	Aug 19	in	n/a	15	175	(9.59?) err
		Sept 02	out				
	middle	Aug 19	in	n/a	14.5	145.1	n/a
		Sept 02	out	0.63	15.4	167.9	8.22
Daphne Island	east	Aug 20	in	0.86	14.6	143.7	7.75
		Sept 03	out	0.2	16.1	187.5	8.41
	west	Aug 20	in	1.05	14.8	166	7.99
		Sept 03	out	0.2	16.1	187.5	8.41
	middle	Aug 20	in	1.14	15	164.3	7.97
		Sept 03	out	0.46	16.2	187.5	8.41
upstream Weyerhaeuser	east	Aug 22	in	0.48	13.6	0.21 mS	n/a
		Sept 05	out	0.41	16.2	00.2 mS	n/a
	west	Aug 22	in	0.65	13.1	0.25 mS	n/a
		Sept 05	out	0.46	16.1	00.1 mS	n/a
Weyerhaeuser	pond	Aug 22	in	mill data	23.8	2.45 mS	8.42
		Sept 05	out		23	2.21 mS	8.74
downstream Weyerhaeuser	east	Aug 22	in	0.6	14	0.23 mS	8.8
		Sept 05	out	0.53	16.6	00.2 mS	n/a
	west	Aug 22	in	0.33	13.2	0.23 mS	8.93
		Sept 05	out	0.46	16.3	00.2 mS	n/a
upstream Daishowa	west	Aug 24	in	0.35	15.5	197	n/a
		Sept 07	out	0.33	n/a	252	n/a
	east	Aug 24	in	0.53	14.2	202	n/a
		Sept 07	out	0.27	n/a	261	n/a

Station	Side	Date	in / out	Veloc. (m/s)	Temp. (C)	Cond. (uS)	pH
Daishowa	pond	Aug 24	in	mill data	33.4	3.59 mS	7.07
		Sept 07	out		31.3	2.9 mS	6.9
downstream Daishowa	west	Aug 24	in	0.06	14.5	187.8	n/a
		Sept 07	out				
	east	Aug 24	in	0.22	14.6	258	n/a
		Sept 07	out	0.12	17	288	n/a

APPENDIX C PLHC-1 EROD activity dose response curves.





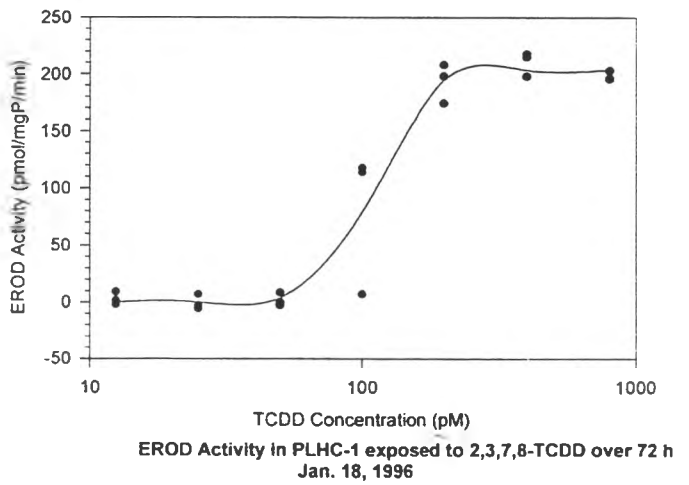


Figure AC.1. EROD activity (pmol/mg/min) of PLHC-1 exposed for 72 h to graded doses of TCDD standard (pM). EC50s (from top to bottom) were 157, 190 and 109 pM.

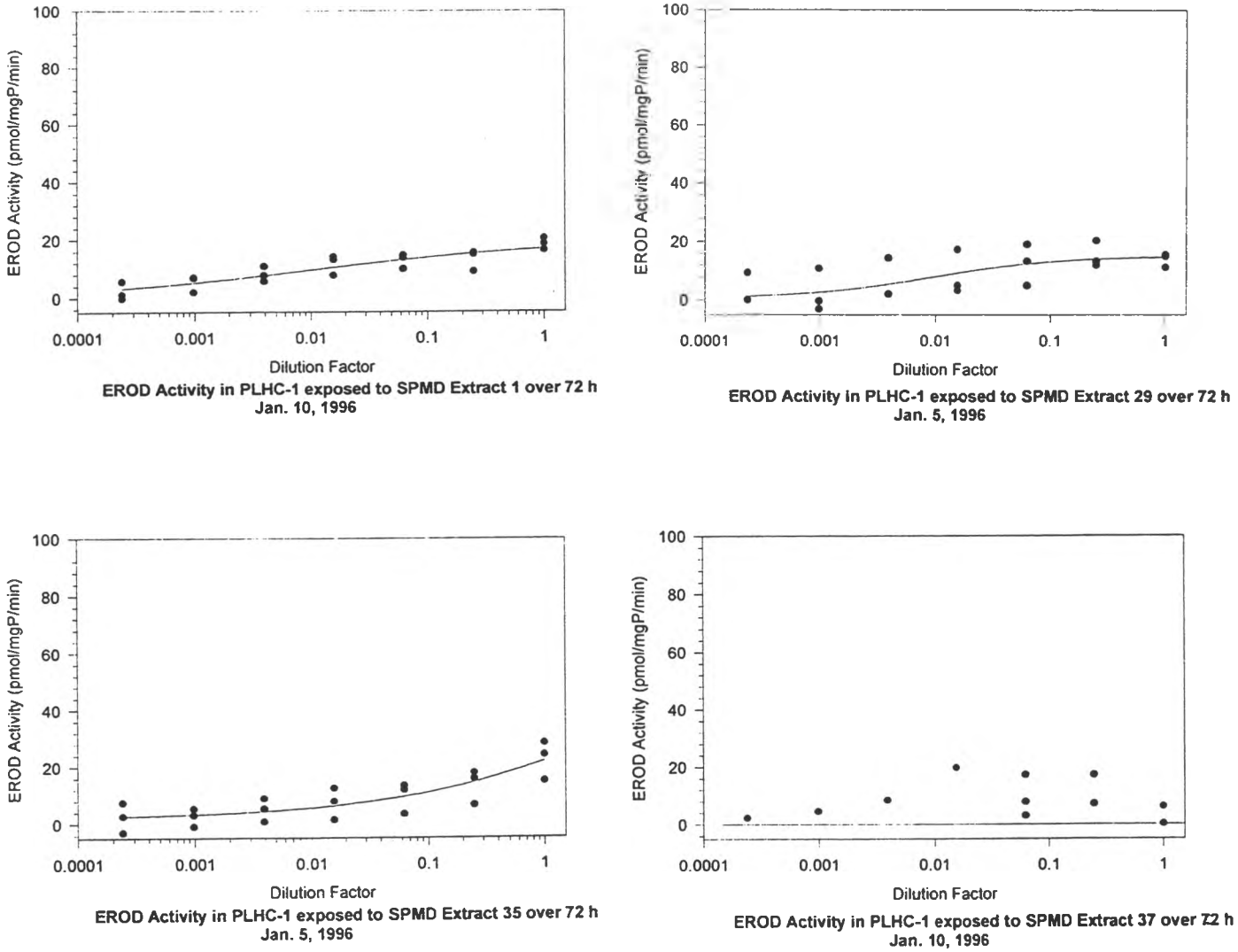
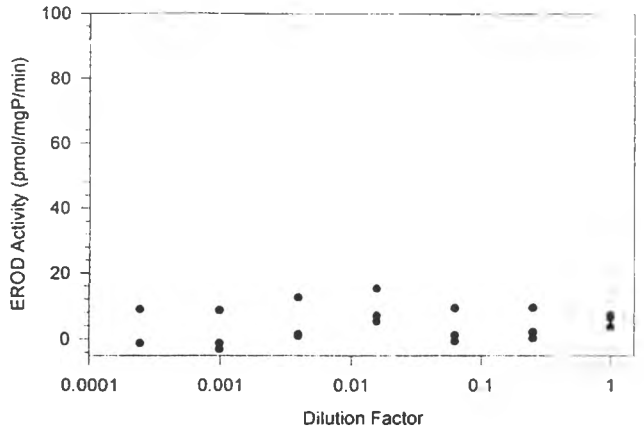
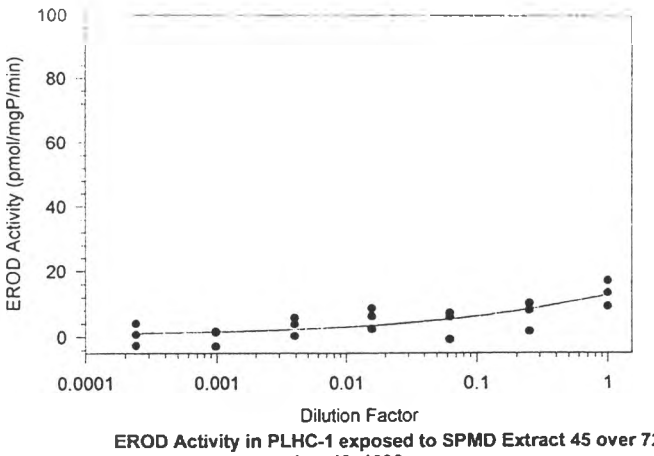


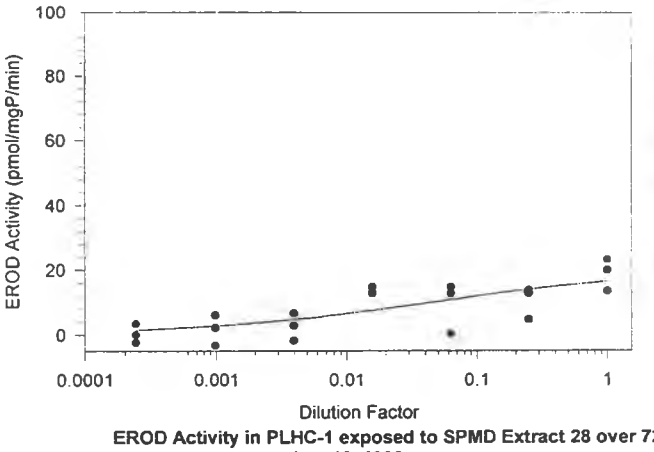
Figure AC.2. EROD activity (pmol/mg/min) of PLHC-1 exposed for 72 h to graded doses of extracts from SPMDs deployed at upstream and downstream (of pulp mill) sites on the Athabasca, Peace and Wapiti Rivers. Samples shown are upstream Alberta Pacific south (top left), downstream Weyerhaeuser east (top right), upstream Daishowa east (bottom left), and downstream Daishowa east (bottom right). Concentrations of SPMD extracts (x axis) indicate relative concentration of extract in isooctane, where 1 is 100 % concentrated extract, 0.1 is 10 % extract, 0.01 is 1 % extract etc.



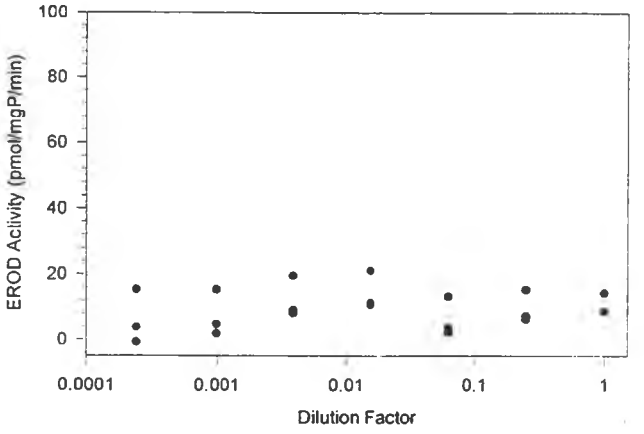
EROD Activity in PLHC-1 exposed to SPMD Extract 44 over 72 h
Jan. 18, 1996



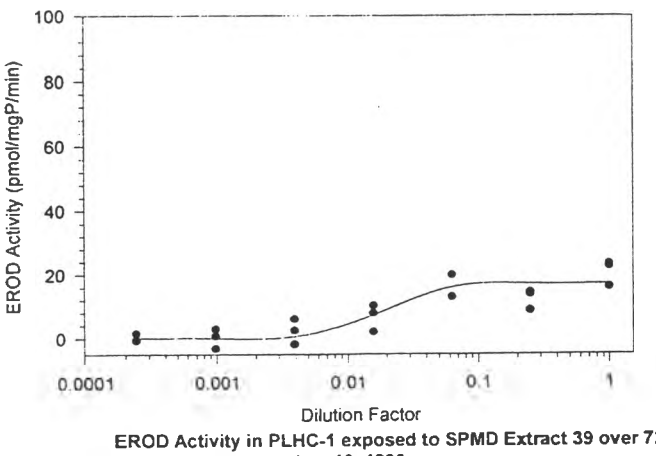
EROD Activity in PLHC-1 exposed to SPMD Extract 45 over 72 h
Jan. 18, 1996



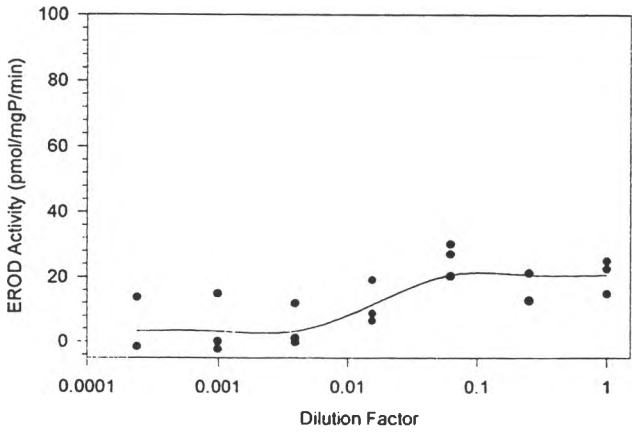
EROD Activity in PLHC-1 exposed to SPMD Extract 28 over 72 h
Jan. 10, 1996



EROD Activity in PLHC-1 exposed to SPMD Extract 43 over 72 h
Jan. 18, 1996



EROD Activity in PLHC-1 exposed to SPMD Extract 39 over 72 h
Jan. 10, 1996



EROD Activity in PLHC-1 exposed to SPMD Extract 40 over 72 h
Jan. 10, 1996

Figure AC.3. EROD activity (pmol/mg/min) of PLHC-1 exposed for 72 h to graded doses of extracts of SPMDs from the secondary treatment ponds of the three pulp mills tested. Dose responses (from top to bottom) are for Alberta Pacific, Weyerhaeuser and Daishowa SPMD extracts. Concentrations of SPMD extracts (x axis) indicate relative concentration of extract in isooctane, where 1 is 100 % concentrated extract, 0.1 is 10 % extract, 0.01 is 1 % extract etc.

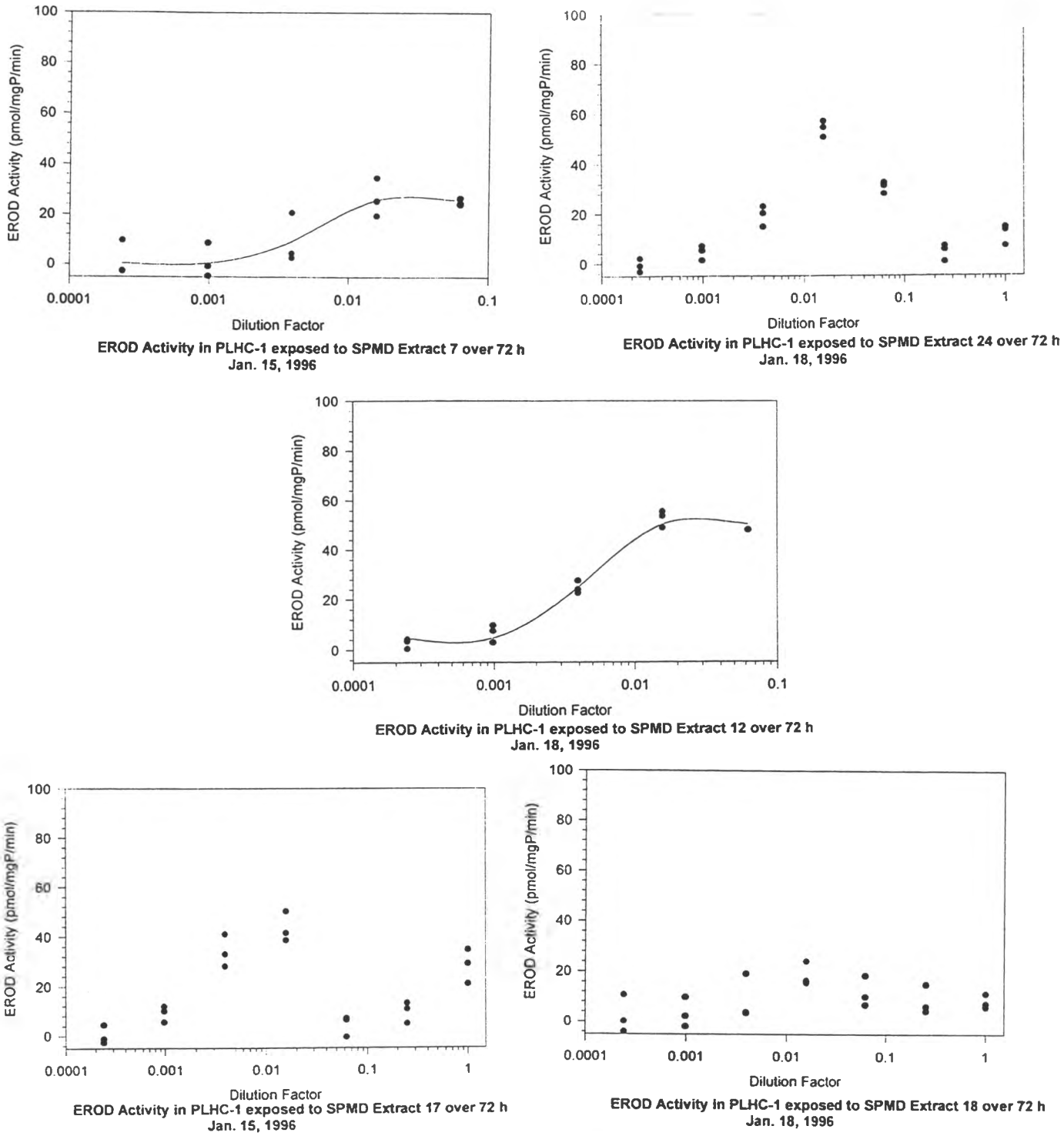
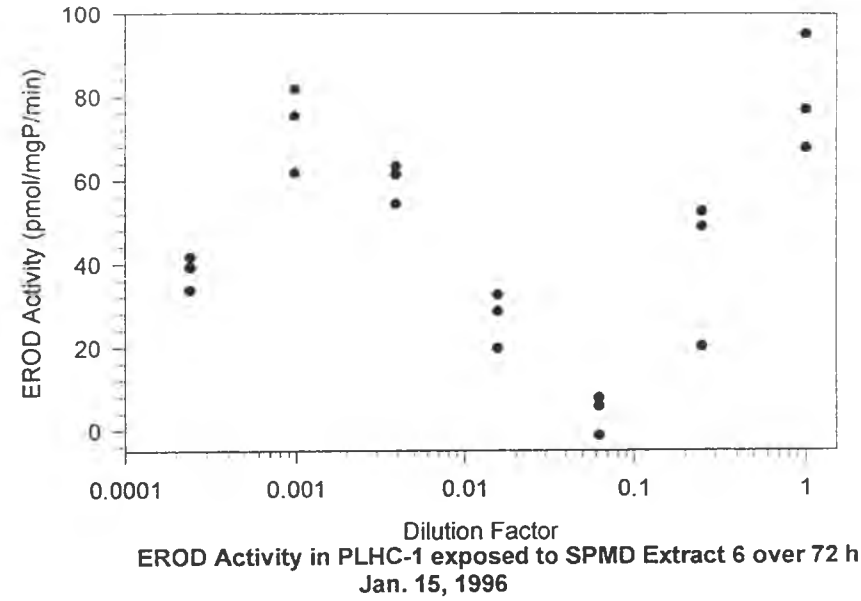


Figure AC.4. EROD activity (pmol/mg/min) of PLHC-1 exposed for 72 h to graded doses of extracts from SPMDs deployed in Athabasca River in the oil sands area. Dose responses are upstream Fort McMurray west (top left), downstream Fort McMurray middle (top right), upstream Suncor east (middle), downstream Suncor east (bottom left), far downstream Suncor middle (bottom right). Concentrations of SPMD extracts (x axis) indicate relative concentration of extract in isooctane, where 1 is 100 % concentrated extract, 0.1 is 10 % extract, 0.01 is 1 % extract etc.



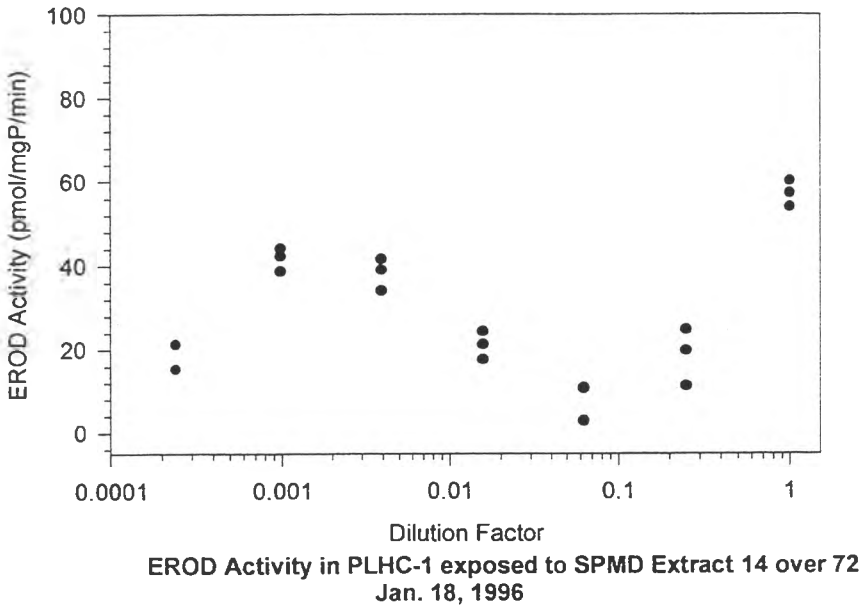


Figure AC.5. EROD activity (pmol/mg/min) of PLHC-1 exposed for 72 h to graded doses of extracts from SPMDs deployed in the Clearwater (south deployment, top figure) and Steepbank Rivers (north deployment, bottom figure). Concentrations of SPMD extracts (x axis) indicate relative concentration of extract in isooctane, where 1 is 100 % concentrated extract, 0.1 is 10 % extract, 0.01 is 1 % extract etc.

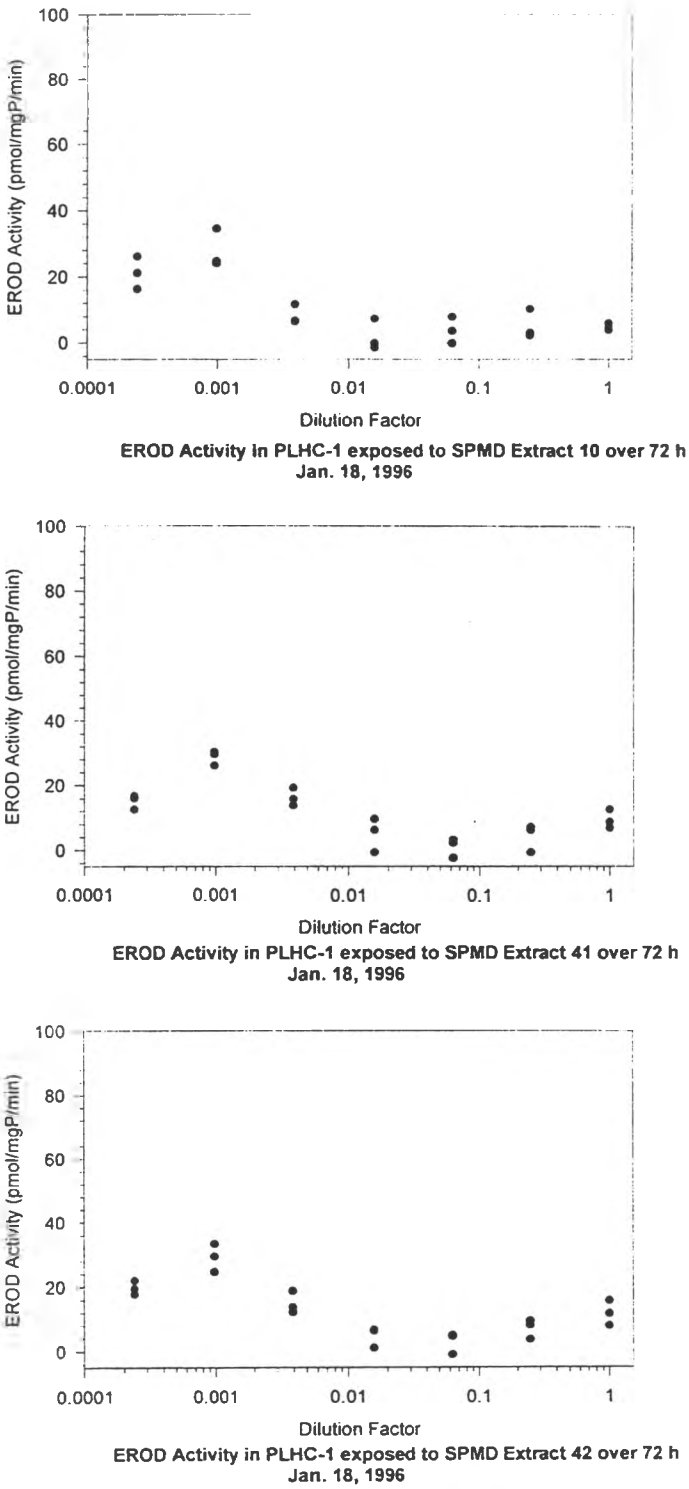


Figure AC.6. EROD activity (pmol/mg/min) of PLHC-1 exposed for 72 h to graded doses of extracts of SPMDs deployed in Suncor wasterwaters. Concentrations of SPMD extracts (x axis) indicate relative concentration of extract in isooctane, where 1 is 100 % concentrated extract, 0.1 is 10 % extract, 0.01 is 1 % extract etc.

APPENDIX D Chemical analyses of SPMD extracts

Table AD. 1. Nominal limits of quantitation* (LOQ) and electron impact ions used for quantitation of PAHs.

Compound	RRF	Sample and standard quantitation ion (m/e)	LOQ (ng)
Naphthalene	authentic	128	4.0
Acenaphthylene	authentic	152	3.5
Acenaphthene	authentic	154	5.5
Fluorene	authentic	166	4.7
Phenanthrene	authentic	178	2.9
Anthracene	authentic	178	3.5
Fluoranthene	authentic	202	2.0
Pyrene	authentic	202	2.0
Benz[a]anthracene	authentic	228	3.5
Chrysene	authentic	228	4.5
Benzo[b]fluoranthene	authentic	252	4.5
Benzo[k]fluoranthene	authentic	252	4.0
Benzo[a]pyrene	authentic	252	4.5
Perylene	authentic	252	3.5
Indeno[1,2,3-cd]pyrene	authentic	276	5.5
Dibenz[a,h]anthracene	authentic	278	5.9
Benzo[g,h,i]perylene	authentic	276	4.8
2-Methylnaphthalene	authentic	142	5.5
1-Methylnaphthalene	authentic	142	5.5
2,6 & 2,7-Dimethylnaphthalene	authentic	156	6.5
1,6-Dimethylnaphthalene	authentic	156	4.8
2,3- & 1,4-Dimethylnaphthalene	authentic	156	5.2
1,5-Dimethylnaphthalene	authentic	156	3.3
1,2-Dimethylnaphthalene	authentic	156	9.0
2,3,6-Trimethylnaphthalene	authentic	170	7.0
2,3,5-Trimethylnaphthalene	authentic	170	5.2
2-Methylphenanthrene	authentic	192	3.9
2-Methylanthracene	authentic	192	5.5
9/4-Methylphenanthrene	1-Methylanthracene	192	3.9
1-Methylphenanthrene	authentic	192	4.5
9-Methylanthracene	authentic	192	5.5
3,6-Dimethylphenanthrene	authentic	206	3.9
9,10-Dimethylanthracene	authentic	206	9.0
2-Methylfluoranthene	authentic	216	4.5

* A final extract volume of 1 mL.

RRF = Relative response factor

authentic (quantitation based on response factor of compound quantitation ion).

LOQ (Limit of Quantitation) represents the nominal amount of analyte that can be statistically discriminated from the averaged background sample noise. The LOQ was based on a 99 % confidence interval and represents the quantity of analyte required to produce a signal three times the sample noise or 10 standard deviations above the sample noise.

ND = no analyte detected above the LOQ.

Table AD.2. AEH sample numbers of 1995 Athabaska River SPMDs.

AEH sample number	Site description	AEH trip blank number
Alberta Pacific Mill		
1	upstream south	1B
2	upstream north	2B
3	downtream south	3B
4	downstream north	4B
44	Mill pond I	39B
45	Mill pond II	40B
Fort McMurray/Suncor/Oil Sands		
5	Clearwater north	5B
6	Clearwater south	6B
7	upstream Fort McMurray west	7B
8	upstream Fort McMurray east	no sample
9	upstream Fort McMurray middle	9B
10	Suncor Pond	10B
41	Suncor Pond	no sample
42	Suncor pond	no sample
11	upstream Suncor west	11B
12	upstream Suncor east	12B
13	upstream Suncor midle	no sample
14	Steepbank north	14B
15 (exposed to air)	Steepbank south	15B
16	downstream-n Suncor west	16B
17	downstream-n Suncor erast	17B
18	downstream-f Suncor east	18B
19	downstream-f Suncor east	19B
no sample	downstream-f Suncor north	no sample
21	downstream-f Suncor middle	no sample
22	downstream-f McMurray east	22B
23	downstream-f McMurray west	23B
24	downstream-f McMurray middle	no sample
25	Daphne Island east	25B
26	Daphne Island west	26B
27	Daphne Island middle	no sample
Weyerhaeuser Mill/Wapiti and Smoky Rivers		
28	Weyerhaeuser pond	28B
43	Weyerhaeuser pond	no sample
29	downstream Weyerhaeuser east	29B
30	downstream Weyerhaeuser west	30B
31	upstream Weyerhaesuer east	31B
32	upstream Weyerhaesuer west	no sample
Daishowa Mill/Peace River		
39	Diashowa Pond	39B
40	Diashowa Pond	40B
34	upstream Daishowa west	no sample
35	upstream Daishowa east	35B
36	downstream Daishowa west	36B
37	downstream Daishowa east	37B
CONTROL	spmd blank	38B
CONTROL	solvent blank	CB

Table AD. 3. Concentrations (ng/mL) of PAH in Blank samples of SPMD dialysates.

Sample name	Solvent blank	Trip blank	Trip blank	Trip blank	Trip blank	Trip blank
AEH Lab number	*10B*	*12B*	*31B*	*39B*	*1B*	*7B*
Compound						
Naphthalene	2.2	960	83	0.87	5000	2700
2-Methylnaphthalene	ND	71	24	0.91	500	230
1-Methylnaphthalene	ND	44	13	0.64	330	150
2,6 & 2,7-Dimethylnaphthalene	6.5	8.1	8.6	3.0	21	11
1,6-Dimethylnaphthalene	ND	4.3	3.8	ND	23	8.8
2,3- & 1,4-Dimethylnaphthalene	ND	1.0	1.3	ND	7.3	3.3
1,5-Dimethylnaphthalene	ND	0.59	0.71	ND	4.1	1.9
1,2-Dimethylnaphthalene	ND	2.8	0.52	ND	14	4.2
2,3,6-Trimethylnaphthalene	ND	2.8	ND	ND	1.7	ND
2,3,5-Trimethylnaphthalene	ND	ND	ND	ND	1.7	ND
Acenaphthylene	1.8	180	78	2.0	1600	780
Acenaphthene	ND	23	7.0	6.7	100	52
Fluorene	ND	23	9.8	7.9	150	60
Phenanthrene	5.6	210	85	40	1400	590
2-Methylphenanthrene	ND	25	6.5	11	64	27
2-Methylanthracene	ND	ND	ND	ND	16	5.2
9/4-Methylphenanthrene	ND	15	4.3	5.1	51	22
1-Methylphenanthrene	ND	12	3.5	4.2	41	18
9-Methylanthracene	ND	8.2	ND	1.6	11	5.9
3,6-Dimethylphenanthrene	ND	4.4	ND	2.7	18	3.9
9,10-Dimethylanthracene	ND	4.0	ND	ND	17	7.7
2-Methylfluoranthene	ND	2.6	ND	ND	20	7.7
Anthracene	ND	4.9	5.0	ND	98	58
Fluoranthene	2.7	71	24	9.2	500	220
Pyrene	2.8	71	26	8.8	520	220
Benz[a]anthracene	ND	0.45	ND	ND	76	25
Chrysene	ND	12	3.6	ND	100	32
Benzo[b,k]fluoranthene	ND	20	ND	ND	480	180
Benzo[a]pyrene	ND	3.8	ND	ND	57	14
Perylene	ND	ND	ND	ND	11	2.7
Indeno[1,2,3-cd]pyrene	ND	7.7	ND	ND	110	5.0
Dibenz[a,h]anthracene	ND	ND	ND	ND	7.7	ND
Benzo[g,h,i]perylene	ND	18	ND	ND	160	38

Table AD. 4. Concentrations (ng/mL) of PAH in Alberta Pacific SPMD dialysates.

Sample name	Upstream S.	Mill pond I	Mill pond II	Downstream S.
AEH Lab number	*1*	*44*	*45*	*3*
Compound				
Naphthalene	26	31	25	70
2-Methylnaphthalene	2.8	6.8	3.9	16
1-Methylnaphthalene	1.6	5.4	2.9	8.4
2,6 & 2,7-Dimethylnaphthalene	15	13	ND	3.9
1,6-Dimethylnaphthalene	ND	3.2	ND	2.7
2,3- & 1,4-Dimethylnaphthalene	ND	ND	ND	ND
1,5-Dimethylnaphthalene	ND	ND	ND	ND
1,2-Dimethylnaphthalene	ND	ND	ND	1.9
2,3,6-Trimethylnaphthalene	ND	2.6	1.1	ND
2,3,5-Trimethylnaphthalene	ND	ND	0.20	ND
Acenaphthylene	2.2	2.2	1.5	3.6
Acenaphthene	9.4	23	11	15
Fluorene	11	61	38	11
Phenanthrene	67	1200	820	76
2-Methylphenanthrene	26	250	170	36
2-Methylanthracene	ND	ND	ND	ND
9/4-Methylphenanthrene	32	170	120	42
1-Methylphenanthrene	26	140	98	34
9-Methylanthracene	51	33	ND	150
3,6-Dimethylphenanthrene	5.2	31	18	6.9
9,10-Dimethylanthracene	ND	ND	ND	ND
2-Methylfluoranthene	3.7	18	16	5.0
Anthracene	0.86	ND	ND	0.57
Fluoranthene	64	760	510	79
Pyrene	74	310	210	95
Benz[a]anthracene	1.4	58	80	3.5
Chrysene	19	360	280	12
Benzo[b,k]fluoranthene	30	54	15	40
Benzo[a]pyrene	ND	20	ND	2.2
Perylene	58	36	ND	26
Indeno[1,2,3-cd]pyrene	ND	ND	ND	ND
Dibenz[a,h]anthracene	ND	ND	ND	ND
Benzo[g,h,i]perylene	ND	52	ND	1.2

Table AD. 5. Concentrations (ng/mL) of PAH in Weyerhaeuser SPMD dialysates.

Sample name	Upstream E.	Mill pond	Mill pond	Downstream E.
AEH Lab number	*31*	*28*	*43*	*29*
Compound				
Naphthalene	57	24	39	38
2-Methylnaphthalene	86	9.2	17	37
1-Methylnaphthalene	36	11	18	14
2,6 & 2,7-Dimethylnaphthalene	35	7.5	17	18
1,6-Dimethylnaphthalene	41	14	32	14
2,3- & 1,4-Dimethylnaphthalene	14	8.2	17	5.8
1,5-Dimethylnaphthalene	7.9	4.7	9.6	3.3
1,2-Dimethylnaphthalene	9.8	4.4	7.1	2.9
2,3,6-Trimethylnaphthalene	17	15	38	15
2,3,5-Trimethylnaphthalene	21	6.8	19	7.4
Acenaphthylene	11	1.3	1.8	3.6
Acenaphthene	11	25	33	5.0
Fluorene	18	66	75	6.4
Phenanthrene	98	180	200	28
2-Methylphenanthrene	61	58	70	14
2-Methylanthracene	7.1	ND	ND	ND
9/4-Methylphenanthrene	63	190	220	19
1-Methylphenanthrene	51	160	180	16
9-Methylanthracene	110	160	190	ND
3,6-Dimethylphenanthrene	12	42	46	6.4
9,10-Dimethylanthracene	ND	1.9	12	ND
2-Methylfluoranthene	5.5	2.6	0.64	ND
Anthracene	4.6	ND	5.5	1.0
Fluoranthene	37	32	34	11
Pyrene	38	50	58	14
Benz[a]anthracene	ND	3.1	2.9	ND
Chrysene	4.2	0.93	ND	4.8
Benzo[b,k]fluoranthene	20	16	17	ND
Benzo[a]pyrene	ND	ND	ND	ND
Perylene	8.3	ND	ND	ND
Indeno[1,2,3-cd]pyrene	ND	ND	ND	ND
Dibenz[a,h]anthracene	ND	ND	ND	ND
Benzo[g,h,i]perylene	ND	ND	ND	ND

Table AD. 6. Concentrations (ng/mL) of PAH in Daishowa SPMD dialysates.

Sample name	Upstream E.	Mill pond	Mill pond	Downstream
AEH Lab number	*35*	*40*	*39*	*37*
Compound				
Naphthalene	110	34	13	52
2-Methylnaphthalene	28	4.8	1.1	4.4
1-Methylnaphthalene	16	9.0	1.0	4.0
2,6 & 2,7-Dimethylnaphthalene	16	3.9	5.1	21
1,6-Dimethylnaphthalene	9.5	9.6	1.9	2.2
2,3- & 1,4-Dimethylnaphthalene	11	3.7	ND	ND
1,5-Dimethylnaphthalene	6.2	2.1	ND	ND
1,2-Dimethylnaphthalene	3.2	3.5	ND	ND
2,3,6-Trimethylnaphthalene	6.1	5.1	ND	0.84
2,3,5-Trimethylnaphthalene	3.6	3.1	ND	ND
Acenaphthylene	8.8	1.7	ND	4.7
Acenaphthene	16	47	1.8	11
Fluorene	27	84	1.4	20
Phenanthrene	210	140	52	160
2-Methylphenanthrene	56	120	ND	36
2-Methylanthracene	ND	6.0	ND	ND
9/4-Methylphenanthrene	120	58	17	51
1-Methylphenanthrene	94	47	14	41
9-Methylanthracene	200	8.9	12	83
3,6-Dimethylphenanthrene	24	22	ND	36
9,10-Dimethylanthracene	ND	ND	ND	ND
2-Methylfluoranthene	11	6.5	2.3	4.7
Anthracene	2.8	ND	ND	ND
Fluoranthene	160	95	100	81
Pyrene	140	140	160	93
Benz[a]anthracene	11	ND	ND	11
Chrysene	240	11	0.17	120
Benzo[b,k]fluoranthene	51	40	36	11
Benzo[a]pyrene	ND	ND	ND	ND
Perylene	220	ND	ND	130
Indeno[1,2,3-cd]pyrene	ND	ND	ND	ND
Dibenz[a,h]anthracene	ND	ND	ND	ND
Benzo[g,h,i]perylene	ND	ND	ND	ND

Table AD. 7. Concentrations (ng/mL) of PAH in Ft. McMurray SPMD dialysates.

Sample name AEH Lab number	Upstream E. *8*	Clearwater *6*	Downstream *23*	Downstream *24*
Compound				
Naphthalene	32	73	19	18
2-Methylnaphthalene	12	72	8.2	3.4
1-Methylnaphthalene	5.0	21	3.1	1.6
2,6 & 2,7-Dimethylnaphthalene	12	67	11	4.3
1,6-Dimethylnaphthalene	6.4	45	4.8	ND
2,3- & 1,4-Dimethylnaphthalene	3.7	54	2.3	ND
1,5-Dimethylnaphthalene	2.1	31	1.3	ND
1,2-Dimethylnaphthalene	ND	15	ND	ND
2,3,6-Trimethylnaphthalene	14	450	2.6	ND
2,3,5-Trimethylnaphthalene	6.2	310	2.9	ND
Acenaphthylene	1.7	52	1.2	0.98
Acenaphthene	7.7	110	5.6	5.4
Fluorene	5.6	39	2.5	ND
Phenanthrene	42	290	17	150
2-Methylphenanthrene	18	710	8.8	36
2-Methylanthracene	ND	320	ND	ND
9/4-Methylphenanthrene	76	1600	17	120
1-Methylphenanthrene	62	1300	14	100
9-Methylanthracene	ND	5.0	ND	82
3,6-Dimethylphenanthrene	16	580	7.8	0.24
9,10-Dimethylanthracene	ND	2500	ND	4.0
2-Methylfluoranthene	7.0	39	0.83	56
Anthracene	ND	1.0	ND	ND
Fluoranthene	42	15	13	210
Pyrene	68	ND	23	610
Benz[a]anthracene	2.9	83	ND	20
Chrysene	50	240	17	530
Benzo[b,k]fluoranthene	9.4	23	4.7	380
Benzo[a]pyrene	ND	370	ND	59
Perylene	35	31	16	270
Indeno[1,2,3-cd]pyrene	ND	ND	ND	34
Dibenz[a,h]anthracene	ND	8.4	ND	4.3
Benzo[g,h,i]perylene	ND	ND	ND	19

Table AD. 8. Concentrations (ng/mL) of PAH in Suncor/Oil Sands SPMD dialysates.

Sample name AEH Lab number	Upstream E. *12*	Suncor pond *10*	Suncor pond *42*	Downstream M. *21*	Steepbank R. *14*	Daphne Is. *26*
Compound						
Naphthalene	43	11	29	87	48	25
2-Methylnaphthalene	20	4.1	6.1	42	24	16
1-Methylnaphthalene	10	0.68	13	17	8.1	8.4
2,6 & 2,7-Dimethylnaphthalene	7.1	18	48	24	18	20
1,6-Dimethylnaphthalene	6.6	2.4	18	20	27	16
2,3- & 1,4-Dimethylnaphthalene	3.2	11	72	13	33	19
1,5-Dimethylnaphthalene	1.8	6.4	41	7.5	19	11
1,2-Dimethylnaphthalene	3.5	78	61	12	38	25
2,3,6-Trimethylnaphthalene	16	75	120	170	830	380
2,3,5-Trimethylnaphthalene	11	96	81	87	410	160
Acenaphthylene	5.1	1.9	ND	4.5	5.5	3.8
Acenaphthene	25	4.8	32	50	190	98
Fluorene	20	1.1	25	23	80	87
Phenanthrene	200	12	ND	160	590	410
2-Methylphenanthrene	370	140	16	330	1700	910
2-Methylanthracene	ND	150	6.5	5.2	ND	ND
9/4-Methylphenanthrene	690	36	28	510	1800	1100
1-Methylphenanthrene	560	29	22	410	1500	890
9-Methylanthracene	180	160	530	78	94	51
3,6-Dimethylphenanthrene	250	18	610	350	580	500
9,10-Dimethylanthracene	29	30	590	180	840	42
2-Methylfluoranthene	42	ND	5.0	45	1.0	55
Anthracene	0.97	16	37	4.6	20	1.3
Fluoranthene	170	830	180	140	360	160
Pyrene	490	40000	23000	370	590	620
Benz[a]anthracene	ND	16	9.3	ND	ND	ND
Chrysene	25	60	18	230	890	39
Benzo[b,k]fluoranthene	100	670	550	65	200	130
Benzo[a]pyrene	ND	1300	760	4.2	ND	4.2
Perylene	220	ND	ND	6.6	17	80
Indeno[1,2,3-cd]pyrene	36	120	6.2	ND	2.7	5.1
Dibenz[a,h]anthracene	ND	64	24	ND	ND	ND
Benzo[g,h,i]perylene	39	1.7	12	ND	ND	8.4

Table AD.9. Mass spectral characterization of chemicals in Athabasca River SPMDs.

Alberta Pacific (*44 and 45*)

1. Nonylphenol.
2. Coumarins (methyl-hydroxy).
3. Anthraquinone.
4. Sulphur
5. Methoxystilbenes.
6. Chlorinated stilbenes.

Daishowa (*39 and 40*)

1. Chlorinated phenols.
2. Chlorinated veratroles.
3. Anthraquinone.
4. Sulphur
5. Methoxystilbenes.
6. Chlorinated stilbenes.
7. Chlorinated diarylethanes.
8. Phthalates.
9. Series of unidentified chlorinated compounds.

Steepbank River (*14*)

1. Cinnolines (methyl and dimethyl).
2. Naphthenic acids (Z =2, 4,6)
3. PAH
4. Methylated phenathrenes (C3 and C4)
5. Phthalates

Suncor (*10 and 42*).

1. Naphthenic acids (Z = 0, 2, 4 and 6).
2. PAH (mainly 3 and 4 ring).
3. Methyl PAH (2 to 5 ring)
4. Benzothiophenes (C1 and C2 methyl analogs).
5. Carbazole (C1 to C5 methyl analogs).
6. Retene (and other substituted phenanthrenes).

Weyerhaeuser (*28 and 43*)

1. Nonylphenol.
2. Coumarins (methyl-hydroxy).
3. Anthraquinone.
4. Sulphur
5. Methoxystilbenes.
6. Chlorinated stilbenes.
7. Chlorinated diarylethanes.
8. Dibenzothiophenes (methyl and dimethyl).

Ft. McMurray (*24*)

1. PAH.
2. Sulphur.
3. Naphthenic acids (Z = C4 and C6)
4. Anthraquinone.

Clearwater River (*6*)

1. Naphthenic acids (Z =2, 4)
3. PAH
4. Methylated phenathrenes (C2 and C3)
5. Phthalates