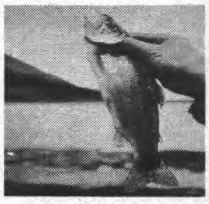
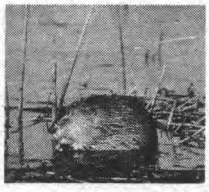
Canada Alberta Northwest Territories

Northern River Basins Study



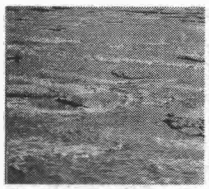


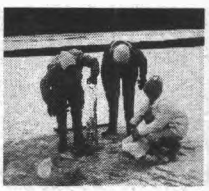





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 83

ACCUMULATION OF FISH MIXED FUNCTION OXYGENASE INDUCERS BY SEMIPERMEABLE MEMBRANE DEVICES IN RIVER WATER AND EFFLUENTS, ATHABASCA RIVER, AUGUST AND SEPTEMBER, 1994



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by

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NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 83

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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.

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

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
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(Dr. P. A. Larkin, Ph.D., Chair) — (Date) 14 Feb 1996

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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, Co-chair) — (Date) 14/02/96

(Robert McLeod, Co-chair) — (Date) 14/02/96

ACCUMULATION OF FISH MIXED FUNCTION OXYGENASE INDUCERS BY SEMIPERMEABLE MEMBRANE DEVICES IN RIVER WATER AND EFFLUENTS, ATHABASCA RIVER, AUGUST AND SEPTEMBER, 1994

STUDY PERSPECTIVE

The aquatic fauna of northern rivers in Alberta are exposed to pulp mill effluent, and other types of industrial and municipal discharges. To understand the risks to fish from industrial effluents discharged into northern 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 contaminants (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). SPMDs are thin polyethylene membrane tubes containing a purified fish lipid (fat). The membrane allows fat soluble chemicals to be absorbed similar to the diffusion of compounds across a fish gill membrane, allowing these devices to be used as surrogate fish. Following a specified time period in a stream, the SPMDs are sent to the laboratory for chemical extraction. After extraction, the compounds are tested for MFO induction on live fish liver cells. Becaues SPMDs can be strategically located in streams and the exposure times controlled, they offer many practical benfits as an initial biodetection tool.

Related Study Questions

1a)	*How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines of 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, sediment 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 along the Athabasca River system that induce MFO activity in fish cell lines. These devices 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. SPMDs were deployed for two weeks at one major town and five industrial wastewater sites (four pulp mills and one oil sands facility) on the Athabasca River, and one pulp mill on the Lesser Slave River.

Forty-five of 68 SPMDs were recovered, with some losses due to fast currents and shifting river bottom. Extracts of SPMDs from three of the four pulp mill effluents tested were two to five times more potent than extracts from SPMDs exposed to background river water upstream of effluent sources. The levels of MFO inducers in SPMDs exposed to river water increased downstream of Fort McMurray. An unknown source of inducers, possibly effluent from the town or from natural erosion of the oil sands, may be a cause for the response. SPMDs deployed in wastewater effluent from Suncor accumulated the highest levels MFO-inducing chemicals, with an induction potency more than 20X that of SPMDs from river water upstream of Suncor.

Results from this study indicate that SPMDs from four pulp mill effluents contained relatively small quantities of MFO inducers, far lower than levels accumulated by SPMDs in a similar study of Ontario pulp mills. By contrast, very high quantities of MFO inducers were accumulated from the Suncor effluent, and from the

Athabasca River upstream of Suncor. Results from this study will serve as the basis for additional research to further verify the apparent trends seen here. In addition to sampling more sites in the field, data will be collected from live fish and SPMDs under controlled laboratory conditions. MFO induction in the laboratory SPMDs will be compared with that from the live fish. Traditional chemical analyses will also be performed on fish tissue and SPMD extracts in an attempt to determine the identity and concentration of accumulated chemicals.

The reader should be aware of the fact that this new technology represents only one tool used to study effects on aquatic environments. There are many other techniques and measures to study ecological integrity and health in these rivers. Results from the next study (2354-E1) using both SPMDs and live fish should give a better understanding of the ecological effects and types of MFO inducing compounds involved, particularly in the Fort McMurray area.
- Science Office

REPORT SUMMARY

Semipermeable Membrane Devices (SPMDs) were deployed for 2 weeks in waters of the Athabasca and Lesser Slave Rivers and in four pulp mill effluents and wastewater from one oil sands mining and upgrading facility. Success of recovery of the SPMDs was 66 %, with loss caused by high water velocity and shifting channels and sediments.

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 "EROD potency equivalents in pg/g".

Extracts of SPMDs from pulp mills were two to five times as potent as extracts of SPMDs exposed to background river water. SPMD extracts from three of the four pulp mill effluents tested (Weldwood, Alberta Newsprint and Slave Lake Pulp) had 62.0, 53.5, and 29.7 pg EROD potency-EQ/g, respectively, significantly more than in Athabasca River water (12.6 pg EROD potency-EQ/g = "background"). SPMDs exposed to effluent from Millar Western (23.0 pg EROD potency-EQ/g) had potencies within the 95 % confidence interval of background.

The levels of MFO induction in SPMDs exposed to river water increased downstream of Fort McMurray. In this area, SPMDs accumulated inducers from the river at levels ranging from 58.5 to 728 pg EROD potency-EQ/g. SPMD accumulation was highly variable, which indicated an unknown source of inducers, possibly an effluent from the town or input from natural erosion of the oil sands.

SPMDs deployed in effluent from Suncor accumulated the most MFO-inducing chemicals (16,800 pg EROD potency-EQ/g), with induction potency over 20 x that of SPMDs from river water upstream of Suncor.

Although this study was preliminary, the results indicated that SPMDs from the four pulp mill effluents contained small quantities of MFO inducers. Compared to MFO induction by extracts of SPMDs deployed in two Ontario bleached kraft mill effluents, the pulp mill effluents from the Athabasca River were one third to one twentieth as potent. By contrast, very high quantities of MFO inducers were accumulated from Suncor effluents. SPMDs deployed in Athabasca River waters downstream of Fort McMurray also contained inducers, indicating some unknown anthropogenic or natural source in this area.

ACKNOWLEDGMENTS

We wish to thank the Northern River Basins Study for support to carry out this study. We also appreciate the cooperation of Weldwood of Canada, Ltd., Millar Western Pulp, Ltd., Alberta Newsprint Company, Ltd., Slave Lake Pulp Corporation, Alberta Pacific and Suncor Inc., Oil Sands Group. The help of Mr. Earl Walker from Technical Operations, NWRI, was greatly appreciated.

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1.0 INTRODUCTION

The purpose of this study was to identify effluents (from five pulp mills and one oil sands mining and upgrading facility) along the Athabasca and Lesser Slave Rivers that contained compounds that induce mixed function oxygenase (MFO) activity in fish.

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 reproduction, growth, pathology and physiology of the fish, 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 breakdown 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.

2.0 MATERIALS AND METHODS

2.1 Study Outline

Locations
Deployment sites were located on the Athabasca and Lesser Slave Rivers, upstream and downstream of 5 pulp mills, one major town and an oil sands mining and upgrading facility. Figure 1 shows a schematic representation of all sites along the Athabasca and Lesser Slave Rivers and Table 1 gives longitude and latitude readings for all SPMD deployment sites.

Sampling
SPMDs installed in triplicate upstream and downstream of each source and in effluent treatment ponds, plus several 'far-field' sites (Table 1). A total of 19 sites were sampled. One deployment device containing two SPMDs was used at upstream sites, one deployment device containing three SPMDs was used inside the pulp mills and oil sands facility, and three replicate deployment devices containing two SPMDs were used at downstream locations. 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.

Dates
The sampling was done on the declining hydrograph during August 21 to September 10, 1994, at water temperatures between 10 and 18 °C.

Measurements
The SPMDs were frozen and returned to the lab, extracted into solvent and split for analysis. The samples were tested for MFO inducing ability with cell culture techniques (a fish cell line).

2.2 Site Descriptions

All SPMD deployment sites were on the Athabasca River or Lesser Slave River (Figure 1). Five pulp mills and one oil sands mining and upgrading facility were chosen for the study (Table 2) and SPMDs were deployed in the final effluents. Upstream sites on the Athabasca River were 2 to 43.5 km above and downstream sites were 7.7 to 28.5 km below the pulp mills or oil sands facility. Some far downstream sites were chosen to determine influence of merging rivers (Lesser Slave River) or large towns (Fort McMurray).

2.3 Sampling Equipment

SPMDs

SPMDs were prepared in clean rooms at the Midwest Science Centre, Columbia, 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 from NWRI labs to the field site at ambient temperatures. 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 deployment was 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 deployment was finished, trip blanks were returned to the sealed can.

Deployment Devices

All metal materials used in the preparation of the SPMD sampling devices were pre-cleaned by immersion in baths of hexane and dichloromethane. The threaded steel rods were pre-treated by spraying with varsol in a degreasing bath, prior to immersion in the two solvent baths. Materials were then wrapped and bagged in clean polyethylene containers for shipment and assembly in the field. 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 30 to 45 cm off the river bottom by a 1 m threaded steel pole set in a 27 kg square patio stone. The aluminum tube could be set at any height from the bottom by adjusting bolts on the threaded rod, and the whole tube assembly was designed to rotate freely in 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 taut and untangled. At one end of the tube, two 1.4 cm holes were drilled through the diameter of the tube to accommodate a 1 m length of 1.3 cm threaded rod. Wire mesh was attached to the open ends of the tube to prevent damage to SPMDs by large pieces of debris. The tube with SPMDs was fixed into place on one end of the rod with locking nuts and a shackle while a pre-drilled 38 cm square concrete patio stone was bolted in place on the other end of the rod. The patio stone was to act as a stable base for the

device when placed in rivers or effluent plumes. The device could be lowered into and retrieved from sampling locations by means of a length of rope attached to the shackle on the top of the rod. In the river installations, a 9 to 12 m length of weighted rope was left attached to the device and allowed to sink downstream of the device. 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 from road bridges over the river or by lowering the device into the river from a small boat. It would have been preferable to deploy the devices using a boat but suitable places from which the boat could be launched were rare. In most cases, where the boat was launched, it involved carrying the boat, motor and all sampling apparatus down a steep slope from the road to the river's edge. At sites upstream of effluent discharge sources, a single deployment device, containing two SPMDs, was deployed, usually in mid-stream. 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, the device was weighted down with steel weights, rather than the patio stone, and suspended by rope or wire cable in the effluent. The only exception to this was the Weldwood Mill at Hinton at which it was possible to deploy the device in exactly the same manner as in the river. All effluent deployments were in flowing channels or ponds, with SPMDs sampling the effluent just before it merged with river water. In mill effluents, one deployment device containing three SPMDs was used. Of the three SPMDs deployed in the mills, two SPMDs were used in the cell line assay, and one SPMD was used to collect a sample for chlorophenol, chloroguaiacol and nonylphenol analyses (data not presented).

Exactly two weeks after being deployed, the deployment devices were retrieved from their locations. To retrieve the devices from river installations, a grapple hook was employed to drag the river bottom until the weighted rope was recovered which then led to the deployment device. 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, sealed in tins purged with purified nitrogen, were frozen for transport back to the labs at NWRI.

2.4 Water Chemistry

Water velocity, conductivity, temperature and pH

At the same time as SPMDs were retrieved, physical measurements and water chemistry samples were taken. The river velocity, water temperature, conductivity and pH were noted. At some locations, it was not possible to take velocity measurements because retrieval was being done from road bridges 10 to 20 m above the water or, as is the case with the mill effluent streams, the electrical conductivity of the effluent was so high that it shorted out the water velocity meter.

Water velocity measurements were obtained using a Price Model 210 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.

2.5 SPMD dialyses and clean up

SPMD containers were maintained at 4 ^{0}C and transported overland to the Midwest Science Centre, Columbia, Missouri. 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 500 mL (1 device) 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), lined with solvent washed tinfoil and capped. The SPMDs 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.

2.6 PLHC-1 Bioassay Methods

SPMD extracts were tested for EROD induction potency in *Poeciliopsis lucida* (top minnow) hepatoma cells (PLHC-1). The PLHC-1 bioassay procedures were a slight modification of the H4IIE bioassay methods for 96-well microtitre plates described in Tillitt *et al.* (1991). The PLHC-1 cells seeded at 20,000 cells/well in 300 µL of D-MEM culture media in 96-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 six 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 potency-EQ) in the samples. TCDD standards were dosed at eight concentrations or doses (0, 0.069, 0.206, 0.617, 1.85, 5.6, 16.7, and 50 pg/well) with each dose replicated four times. Six TCDD curves were analyzed in the PLHC-1 bioassay on that day.

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). The reactions were initiated with 10 μL of 10 mM NADPH (0.5 mM) and allowed to proceed for 10 minutes of kinetic analysis in the fluorometric plate reader (Cytofluor 2300, Millipore Corp.). Resorufin production was measured kinetically, once/minute for 10 min, with an excitation filter wavelength centred at 530 nm and an emission filter wavelength centred at 595 nm.

The relative fluorescence intensity of the samples was then compared to a quadratic fit of an eight point resorufin standard curve (twelve replicates/concentration) and the relative intensity units were converted to pmol resorufin. Resorufin in each well was plotted against time to observe any deviations from linearity of the reaction. A linear regression was then performed on the data from each well to determine an ethoxyresorufin-O-deethylase (EROD) rate (pmol/min) from the slope of the linear regression line along with it's associated estimates of variance. The amount of protein in each well was determined by the Bradford assay and the values used to normalize dose to each well and EROD activity. The doses of each sample (g-equivalents/mg cellular protein) or TCDD standards (pg TCDD/mg cellular protein) were plotted against EROD activity (pmol/min/mg cellular protein) to develop dose-response curves. The linear portions of these curves were used to compare the relative potencies of the samples with that of the standard, TCDD. The determination of EROD potency-equivalents (EROD potency-EQ) was by slope ratio assay (Finney, 1978) as previously described (Ankley *et al.* 1991). Variance estimates were based on an additive model of variance (Finney, 1978) and were calculated as previously described (Tillitt *et al.* 1991, Ankley *et al.* 1991).

The expression of potency of the SPMD extracts as pg EROD potency-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 potency-EQ (pg/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 potency-EQ pg/g SPMD to pg/g triolein, the pg/g SPMD results would be multiplied by five, giving pg/g triolein (example: EROD potency-EQ of 200 pg/g SPMD = EROD potency-EQ of 1,000 pg/g triolein).

3.0 RESULTS

3.1 Physical measurements

Water Velocity
Water velocity ranged from 0.156 m/sec to 1.56 m/sec (Table 3). Average velocity was 0.84 m/sec (standard deviation = 0.305, n = 21). Water velocity changed over the course of the river, and within each site, depending on the side of the river sampled. The overlapped bars of Figure 3 indicate three water velocity readings taken (side, midstream and other side) at one location. In some locations river velocity doubled depending on the side of the river sampled.

Temperature, pH and conductivity
Athabasca River water temperature ranged from 10 to 18 °C (Table 4). The average temperature (± standard deviation) was 16.7 °C (±1.3, n =12) on deployment in August and 14.4 °C (±2.2, n =11) on retrieval in September. Temperatures of the effluents of the five pulp mills were elevated over river water, and ranged from 21.4 to 34.4°C (Figure 4). Suncor's effluent ponds were about 26 °C.

The pH of river water ranged from 7.9 to 8.6 (Table 4). The average pH of Athabasca River water was 8.37 (±0.17, n =12) on deployment and 8.49 (±0.12, n =11) on retrieval. The pH of the pulp mill effluents ranged from 7.7 to 8.6, with the effluent from the Suncor oil sands mining and upgrading facility at a pH of 8.4 (Figure 5). The lower pH's of the effluents relative to Athabasca River water appeared not to influence pH at downstream sites, as there was little change in pH downstream of the pulp mills and oil refinery versus upstream.

Conductivity of Athabasca River water ranged from 140 to 240 μS (Table 4). The average conductivity was 201 μS (±28, n =12) on deployment and 209 μS (±26, n =11) on retrieval. Conductivity of the effluents from the five pulp mills ranged from 1150 to 7900 μS, while Suncor had a lower conductivity of about 720 μS (Figure 6). There was little influence of the elevated conductivities of the effluents on the Athabasca River water, as there was no change in conductivity downstream versus upstream.

3.2 SPMDs

Recovery
Forty-five out of 68 SPMDs were recovered (Table 5). Most of the losses of SPMDs were due to the high velocity of river water and shifting sand of the river bottom. Some SPMDs deployment devices were unable to stand the extreme currents, as evidenced by worn metal joints and torn and worn aluminum tubes. In some deployment areas, the depth of the river changed between deployment and retrieval, so it is likely that SPMDs were buried under the shifting sediments. SPMDs were lost from one pulp mill, the Alberta Pacific Mill in Boyle. Draining and searching the effluent ponds failed to recover the devices.

Method Blanks
There was no detectable induction in *Poeciliopsis lucida* hepatoma cells (PLHC-1) exposed to hexane

that had been extracted in the SPMD dialyses jar, concentrated and taken through all SPMD clean-up and concentration steps.

MFO induction in PLHC-1

Extracts of all SPMDs induced ethoxyresorufin-O-deethylase (EROD) in PLHC-1 cells. Induction potency was expressed as pg EROD potency equivalents per gram SPMD (pg EROD potency-EQ/g). This method of comparison of potencies related the induction seen when fish cells were exposed to graded doses of the SPMD extracts to that seen when cells were exposed to doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The expression of results as pg TCDD-EQ/g does not imply that the SPMD extracts contained TCDD or other chlorinated dioxins or furans. It is simply a reference point to compare the relative potencies of the SPMD extracts.

To determine the amount of contamination present in the SPMDs at the start of the exposure, and to quantify contamination of SPMDs during handling on deployment, trip blanks were used. Trip blanks, handled in the same manner as deployed SPMDs, had from 2.7 pg EROD potency-EQ/g to 23.9 pg EROD potency-EQ/g, though all but one contained less than 10.5 pg EROD potency-EQ/g (Table 6). Average pg EROD potency-EQ/g (± standard deviation) were 6.9 (± 5.25, N=15) for all trip blanks or 5.7 (± 2.74, N=14) pg EROD potency-EQ/g with the one very high trip blank outlier (23.9 pg EROD potency-EQ/g) removed.

At all four pulp mills, SPMD extracts exposed to effluent showed higher induction than those exposed to river water, which contained on average 12.6 pg EROD potency-EQ/g (± 5.6, n = 9) (Table 6). This average background induction level for Athabasca River water was calculated using the upstream and downstream induction from Hinton to Boyle (downstream of Alberta Pacific). Downstream sites north of Fort McMurray were not included in the calculation of the average Athabasca River background level as these SPMDs appeared to be contaminated by some local source (see discussion).

From these data, upper 95 % confidence interval (CI) for the background level of induction in the Athabasca upstream of Fort McMurray was 25.2 pg EROD potency-EQ/g. Effluents from three of the four pulp mills (Weldwood, Alberta Newsprint and Slave Lake Pulp) had induction higher than the 95 % CI for background Athabasca River water (Figure 7), with highest levels of induction only about 5 x the average background. SPMDs deployed in effluent from Millar Western had 23.0 pg EROD potency-EQ/g, which was within the 95 % CI for background induction.

SPMDs from Athabasca River water below Fort McMurray exhibited higher levels of induction compared to the average background upstream (from Hinton to Boyle). Induction was also quite variable: SPMDs deployed at three sites across the river gave induction of 58.5, 728 and 178 pg EROD potency-EQ/g.

SPMDs deployed in the Suncor wastewater pond gave the highest induction, 16,800 pg EROD potency-EQ/g. This was 23 x the highest level of induction seen upstream of Suncor. Downstream of Suncor, SPMDs had elevated concentrations of inducers (309 pg EROD potency-EQ/g) compared to background concentrations in the Athabasca River from Hinton to Boyle. However, these elevated concentrations

in SPMDs downstream of Suncor were no different to those from the site between Fort McMurray and Suncor.

4.0 DISCUSSION

The SPMD sampling was a success, with 66 % of SPMDs recovered after 2 weeks deployment. Most losses of SPMDs did not affect the design of the experiment, but reduced the number of replicate samples. One serious loss was that of the SPMDs from the effluent ponds of Alberta Pacific. This reduced the number of pulp mills examined in the study from five to four.

All SPMD extracts induced EROD in a fish liver cell line, PLHC-1. Trip blanks were SPMDs handled and treated in a similar fashion to deployed SPMDs, but not put in the water. Levels of inducers in trip blanks were low, usually about 6 pg EROD potency-EQ/g. These low levels indicate that MFO inducers had contaminated SPMDs during preparation and during handling and storage in the field.

Background levels of inducers in SPMDs from Athabasca River water were fairly consistent from Hinton to Boyle, averaging about 13 pg EROD potency-EQ/g. Induction measured in SPMDs from this area was low, with levels averaging only twice that of the trip blanks. Background levels of induction were elevated in SPMDs north of Fort McMurray, and highly variable in the area of the oil sands. This may be caused by inducers from sewage or other outfalls from the town of Fort McMurray or from natural oil seeps from the oil sands. The variability in the potency of SPMD extracts from different locations across the river downstream of Fort McMurray indicates local contamination from seepage or from a discrete plume that contacted the SPMDs at only one location on the river.

Of four pulp mills tested, SPMDs from three effluents had induction potencies that were greater than the upper 95 % CI for background levels of induction in Athabasca River water. Of the pulp mill effluents, the most potent SPMD extracts were from Weldwood, followed by Alberta Newsprint and Slave Lake Pulp. SPMDs from these pulp mills contained two to five times the average levels of inducers in SPMDs deployed in the Athabasca River. SPMDs deployed in Millar Western effluent did not induce MFO above the upper 95 % CI for background water.

By contrast, SPMDs deployed for 14 d in wastewater from Suncor had very high levels of MFO inducers, 16,800 pg EROD potency-EQ/g. The potency of extracts of SPMDs from Suncor effluent was over twenty times that of SPMDs from waters upstream of Suncor.

The units of expressing potency of the SPMD extracts were based on comparisons to 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.

The accumulation of compounds within SPMDs is complex, and related to the compound's K_{ow} (octanol water partition coefficient) and molecular size. SPMD uptake rate can be expressed as the litres of water sampled by the SPMD per day. For naphthalene the rate of sampling is about 0.3 L/day, and the time to reach 90 % of equilibrium concentrations is 7 d (Huckins *et al.* 1995a). The low sampling rate reflects

the low K_{ow} of naphthalene: The small size of the molecule allows easy passage through the membrane pores, but the limited solubility in triolein means equilibrium is reached rapidly. Phenanthrene requires longer (21 d) to reach equilibrium, and the sampling rate is about 4 L/day (Huckins *et al.* 1995a).

The higher the K_{ow}, the more compound will accumulate in the lipid, and the longer it will take for the concentration in the lipid to reach equilibrium with the concentration in the water. SPMDs concentrate chrysene and pyrene from water at a rate of about 5 to 6 L/day (Huckins *et al.* 1995a). For compounds larger than chrysene and pyrene (4 aromatic rings), size becomes a limiting factor. The high K_{ow} favours accumulation of the compounds in the triolein, but the size of the molecules impedes rapid transfer through pores in the polyethylene membrane, and accumulation is slowed. SPMDs concentrate large PAHs, such as benzo[a]pyrene and benzo[g,h,i]perylene, at rates of about 3.7 and 2.2 L/day, respectively (Huckins *et al.* 1995a).

Because of the long time to reach equilibrium, over 21 days for PAHs larger than phenanthrene, the MFO inducer(s) concentrated by the SPMDs in this study were probably not in equilibrium with water concentrations. Had the SPMDs been deployed for 40 or 50 days, induction potency may have been greater, and possibly the unknown inducing compound(s) would have reached equilibrium. The 14 day deployments in this study assured the SPMDs were still in the linear portion of their uptake phase, thus comparisons could be made between studies. For example, potencies of SPMDs deployed for 6 or 7 days in Ontario pulp mill effluents could be doubled to roughly compare with potencies of Athabasca River SPMDs deployed for 14 days. SPMDs from this study were not in equilibrium with either the river waters or effluents, but this should not affect conclusions about relative potencies.

Fouling of the membrane will also affect sampling rate, but effects are not as dramatic as expected after visual examination of fouled membranes. Huckins *et al.* (1995a) left SPMDs in the Upper Mississippi for 58 d, and then removed the fouled membranes to laboratory water to study their uptake properties. Uptake of phenanthrene in the fouled membranes was found to be 35 % less than uptake into unfouled membranes. Fouling should have slowed the uptake of compounds from the Athabasca River and effluents, as the SPMDs were left for 14 d and had a thin film of growth covering them. The effect of the slight fouling of the membranes would be an underestimation of the potency of the effluents and wastewaters, but the degree of underestimation is unknown.

Flow and current of effluent over the SPMD varied at each deployment site along the Athabasca River. The water velocity past the membrane does not influence the concentrations of inducers in the SPMDs, as the rate-limiting step for uptake of compounds into the SPMDs is membrane transfer (Huckins *et al.* 1995a). Water flow past the SPMDs was many fold greater than the highest sampling rates (about 6 L/day) reported for the four ring PAHs (Huckins *et al.* 1995a), so site to site differences in flow should not have affected SPMD uptake of inducers from the effluents and wastewaters.

Water temperatures varied between SPMD deployment sites. River temperatures were 10 to 17 °C, while effluent and wastewaters were 20 to 33 °C. Temperature can affect uptake of certain classes of chemicals across the polyethylene membrane. Huckins *et al.* (1995a) found a 1.3 to 2 fold increase in rate of uptake of organochlorine pesticides with every 5 °C increase in temperature. They theorized the

dramatic increase in uptake rate with increased temperature is because higher temperatures may increase the molecular diffusion and polymer free volume, thereby permitting the pesticides to more easily enter the SPMD membrane pores. However, the influence of temperature on diffusing chemicals and the membrane is not consistent. For more rigidly-structured molecules, such as PAHs, the influence of temperature is minimal. Huckins *et al.* (1995a, b) found very little effect of increased temperatures on the uptake of priority PAHs by SPMDs.

Temperature may have affected SPMD uptake of MFO-inducers in this study, increasing the uptake with increased water temperature. However, the types of compounds that cause MFO induction, dioxins, furans and PAHs, are relatively planar and rigid molecules, and so should not be affected as much by increased temperature, compared to less planar and less rigid molecules such as the organochlorine pesticides. Based on the work of Huckins *et al.* (1995a, b) we would not expect a dramatic effect of temperature on the uptake of MFO-inducing compounds from effluents and Athabasca River waters by SPMDs.

In pulp mill effluents and in effluent from Suncor, it is unknown which compounds in the SPMDs are causing MFO induction. However we can roughly estimate sampling rates of about 4 to 6 L/day for three to five ring compounds. This means that the 16,800 pg EROD potency-EQ/g SPMD accumulated in Suncor wastewater represents about 1,200 pg TCDD equivalents/L wastewater (16,800 pg EROD potency-EQ/g SPMD x 5 g SPMD/ (14 d x 5 L/day)). If we assume the unknown MFO-active substance in Suncor wastewater is a less potent MFO inducer than TCDD, the concentrations of the compound(s) in wastewater should be above 1.2 ng/L.

There is very little data comparing the uptake of compounds into SPMDs with their uptake rates in fish. Huckins *et al.* (1990) compared data for SPMD uptake of 2,2',5,5'-tetrachlorobiphenyl (2,2',5,5'-TCB) with that of Bruggeman *et al.* (1981) who examined uptake of this compound in goldfish (*Carassius auratus*). Concentrations in SPMDs relative to water concentration of 2,2',5,5'-TCB were about 2 times those in goldfish for the first part of the exposure, but after 20 d the concentration factors for goldfish exceeded the SPMDs. Special SPMDs that were formulated to contain lipid extracted from grass carp (*Ctenopharyngodon idella*) rather than triolein, paralleled the goldfish concentration for the first 7 d of exposure, after which the goldfish concentration factor for 2,2',5,5'-TCB (compared to water concentrations) exceeded the concentration factor for the carp-lipid-containing SPMDs (Huckins *et al.* 1990). More research is required to assess uptake of compounds from water by SPMDs and fish. We are planning simultaneous fish and SPMD exposures to pulp mill effluents and oil sands wastewaters, to examine uptake of compounds and MFO induction in live fish and fish cells exposed to SPMD extracts.

Typical levels of induction seen in fish cells exposed to extracts of SPMDs deployed for 6 to 7 d in effluent from bleached kraft mills in Ontario are in the range of 500 to 1200 pg EROD potency-EQ/g triolein (field and lab studies, Parrott *et al.* 1994). The calculation for the SPMDs in the Athabasca study was done using the entire weight of the SPMD, which was the weight of the triolein, about 1 g, plus the weight of the polyethylene membrane, about 4 g. If we express the levels of induction seen in pulp mills in Ontario as the full weight of the SPMD, typical concentrations of inducers found in SPMDs from

Ontario pulp mills would be about 100 to 240 pg EROD potency-EQ/g SPMD. These amounts were accumulated in half the time of those from the present study. Correcting for time, the typical concentrations accumulated from typical Ontario pulp mill effluents in 12 to 14 d would be about 200 to 480 pg EROD potency-EQ/g SPMD.

Compared to typical potencies of extracts of SPMDs deployed in Ontario pulp mill effluents, the pulp mill effluents from the Athabasca River appear to contain lower concentrations of inducing chemicals (accumulating 23 to 62 pg EROD potency-EQ/g SPMD in 14 d compared to approximately 200 to 480 pg EROD potency-EQ/g SPMD accumulated from bleached kraft mill effluents in Ontario). SPMDs deployed in Athabasca pulp mill effluents accumulated one third to one twentieth the EROD potency-EQ of SPMDs from Ontario pulp mill effluents. However, the Suncor effluent appears to contain high concentrations of inducers, with induction potency of SPMD extracts over thirty times the highest concentrations seen in pulp mills from Ontario.

Although the data collected in this summary were preliminary, they indicate that effluents from pulp mills on the Athabasca contained very low amounts of MFO inducers. By comparison, SPMDs from oil refinery wastewaters contained high levels of MFO inducing chemicals. In addition, inducers from natural or anthropogenic sources were present in SPMDs from waters around the area of Fort McMurray. To be sure of the trends seen in the data, the study should be repeated at several times of year, with more replicate SPMDs at each site, and with a knowledge of effluent discharge rates at each industry site.

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Table 1. Location of SPMD deployment sites on Athabasca and Lesser Slave Rivers, with longitude and latitude measurements. Distance (km) from each pulp mill or oil refinery is shown, with negative numbers indicating upstream sites and positive numbers indicating downstream sites.

Location	Distance from Mill (km)	Latitude deg.	min.	sec.	Longitude deg.	min.	sec.
Hinton upstream, at Maskuta Creek	-7.0	53	22	50	117	39	0
Weldwood mill in Hinton	0.0	53	25	15	117	34	15
Hinton downstream, at Obed Mountain Bridge	36.0	53	37	20	117	12	30
Whitecourt, upstream of ANC, at Windfall Bridge	-20.0	54	12	25	116	3	30
Alberta Newsprint mill, northeast of Whitecourt	0.0	54	10	25	115	48	45
Whitecourt, upstream of Millar Western	7.7/-2.0	54	9	10	115	43	0
Millar Western mill in Whitecourt	0.0	54	9	0	115	41	15
Whitecourt downstream of M-W, at Blue Ridge	22.0	54	9	40	115	23	25
Lesser Slave R., upstream of Slave Lake Pulp	-2.0	55	17	30	114	35	30
Slave Lake Pulp mill	0.0	55	15	30	114	35	0
Lesser Slave R., downstream of Slave Lake Pulp	15.0	55	16	0	114	20	0
Athabasca R. below confluence of Lesser Slave R.	38.0	55	10	10	114	2	30
Athabasca R., upstream of Al-Pac, at Athabasca	-43.5	54	43	40	113	16	20
Alberta-Pacific mill, north of Boyle	0.0	54	56	0	112	52	0
Athabasca R.,downstream of Al-Pac, at Calling R.	28.5	55	5	30	112	52	40
Upstream of Fort McMurray	-33.0	56	44	55	111	23	45
Downstream of Fort McMurray, at mile 11	-20.1	56	50	30	111	24	10
Suncor plant, north of Fort McMurray	0.0	57	0	15	111	28	30
Downstream of Oil Sands projects, at mile 33	15.3	57	6	55	111	35	15

Table 2. Description of the five pulp mills and one oil sands mining and upgrading facility. SPMDs were deployed upstream and downstream of each location, as well as in final effluent.

Source	Type of Pulp Mill	Type of Wood
Pulp Mills		
Weldwood of Canada, Ltd.	Bleached kraft pulp mill Also treat municipal sewage for Hinton residents (10,000)	softwoods
Alberta Newsprint Company, Ltd.	Thermo-mechanical pulp mill (sodium hydrosulphite for brightening agent)	softwoods (spruce and lodgepole pine), also recycled newspaper (about 8 % of fibre supply)
Millar Western Pulp, Ltd.	Bleached-chemi-thermo-mechanical pulp mill (alkaline peroxide for bleaching)	softwoods: aspen, spruce and lodgepole pine
Slave Lake Pulp Corporation	Bleached-chemi-thermo-mechanical pulp mill (hydrogen peroxide for bleaching)	softwoods, mostly aspen
Alberta Pacific	Bleached kraft pulp mill (chlorine dioxide for bleaching)	hardwoods and softwoods

Source	Type of Process
Oil Refinery	
Suncor Inc., Oil Sands Group	Hot water used to remove bitumen from mined oil sands, but these tailings (composed of bitumen, sand and fine clay particles) are contained on site. The effluent stream is actually a refinery wastewater from the oil upgrader.

Table 3. Water velocity measurements (revolutions/40 sec and m/sec) at sites on the Athabasca and Lesser Slave Rivers where SPMDs were deployed, Aug 21 to Sept 10, 1994.

Location	Date	Water velocity Measurements		
		Site Position	Revs/40 sec	Velocity (m/sec)
Hinton upstream, at Maskuta Creek	21-8-94	mid	70	1.207
Downstream of Fort McMurray, at mile 11	27-8-94	east	91	1.548
		mid	45	0.776
		west	25	0.436
Downstream of Oil Sands projects, at mile 33	27-8-94	east	40	0.686
		mid	54	0.934
		west	47	0.815
Upstream of Fort McMurray	27-8-94	mid	57	0.985
Hinton upstream, at Maskuta Creek	4-9-94	mid	32	0.560
Hinton downstream, at Obed Mountain Bridge	4-9-94	south	46	0.803
		mid	75	1.287
		north	89	1.555
Weldwood Mill in Hinton	5-9-94		N/D[a]	
Whitecourt, upstream of Millar-Western	5-9-94		56	0.973
Whitecourt downstream of M-W, at Blue Ridge	5-9-94	south	55	0.946
		mid	42	0.725
		north	51	0.895
Millar-Western mill in Whitecourt	6-9-94		N/D[a]	
Alberta Newsprint mill, northeast of Whitecourt	6-9-94		N/D[a]	
Whitecourt, upstream of ANC, at Windfall Bridge	6-9-94		N/D[b]	
Lesser Slave R., upstream of Slave Lake Pulp	6-9-94		47	0.815
Slave Lake Pulp mill	7-9-94		N/D[a]	
Lesser Slave R., downstream of Slave Lake Pulp	7-9-94	south	34	0.594
		mid	51	0.895
		north	54	0.934

Location	Date	Water velocity Measurements		
		Site Position	Revs/40 sec	Velocity (m/sec)
Athabasca R. below confluence of Lesser Slave R.	7-9-94		N/D	
Athabasca R., upstream of Al-Pac, at Athabasca	7-9-94		33	0.555
Alberta-Pacific mill, north of Boyle	8-9-94		N/D[a]	
Athabasca R., downstream of Al-Pac, at Calling R.	8-9-94	east	51	0.895
		mid	60	1.027
		west	30	0.516
Suncor plant, north of Fort McMurray	9-9-94		N/D[b]	
Upstream of Fort McMurray	9-9-94		51	0.895
Downstream of Fort McMurray, at mile 11	10-9-94	east	77	1.326
		mid	29	0.514
		west	8	0.156
Downstream of Oil Sands projects, at mile 33	10-9-94	east	N/D	
		mid	43	0.725
		west	N/D	

N/D = not done
[a] = effluent conductivity too high - shorted out water velocity device
[b] = sampled from high bridge
[c] = not done due to company safety regulations

Table 4. Water chemistry measurements (temperature, °C (temp.), pH, conductivity, μS (cond.)) at sites on Athabasca and Lesser Slave Rivers where SPMDs were deployed, Aug 21 to Sept 10, 1994.

	SPMD Deployment				SPMD Retrieval			
Site	date	temp.	pH	cond.	date	temp.	pH	cond.
Upstream	Aug 21/94	14.6	8.55	140	Sep 4/94	10.3	8.59	202
Weldwood	Aug 22/94	32.2	8.14	1430	Sep 5/94	25.9	8.12	1670
Downstream South	Aug 21/94	15.5	8.37	177	Sep 4/94	11.1	8.5	187
Mid-stream	Aug 21/94	15.6	8.41	176	Sep 4/94	11.1	8.49	202
North	Aug 21/94	15.6	8.4	178	Sep 4/94	11	8.51	185
Upstream	Aug 23/94	13.9	8.43	199	Sep 6/94	13.2	8.67	214
Alberta Newsprint	Aug 23/94	31.8	7.72	1200	Sep 6/94	27.9	7.68	1151
Upstream	Aug 22/94	16.2	8.47	225	Sep 5/94	13.9	8.54	224
Millar Western	Aug 23/94	34.4	8.24	7900	Sep 6/94	33.8	8.21	7570
Downstream South	Aug 22/94	16.6	8.45	209	Sep 5/94	15	8.51	244
Mid-stream	Aug 22/94	16.6	8.4	211	Sep 5/94	14.8	8.54	227
North	Aug 22/94	16.7	8.43	219	Sep 5/94	14.8	8.55	226
Upstream	Aug 23/94	17.5	8.1	173.5	Sep 6/94	16.2	8.24	164
Slave Lake Pulp	Aug 24/94	31.4	8.59	4450	Sep 7/94	29.1	8.44	2250
Downstream South	Aug 24/94	16.9	7.97	162	Sep 7/94	16	8.35	162
Mid-stream	Aug 24/94	16.9	8.05	174	Sep 7/94	16	8.32	160

	SPMD Deployment				SPMD Retrieval			
Site	date	temp.	pH	cond.	date	temp.	pH	cond.
North	Aug 24/94	16.7	7.94	171	Sep 7/94	16.1	8.28	160
Ath. below conf L. Slave	Aug 24/94	17.5	8.38	234	Sep 7/94	N/D	N/D	N/D
Upstream	Aug 24/94	18.2	8.33	222	Sep 7/94	17.3	8.44	241
Alberta-Pacific	Aug 25/94	21.4	7.99	1718	Sep 8/94	22.6	7.92	1158
Downstream East	Aug 25/94	17.4	8.31	209	Sep 8/94	17.1	8.44	234
Mid-stream	Aug 25/94	17.7	8.3	220	Sep 8/94	17.1	8.41	232
West	Aug 25/94	17.5	8.29	210	Sep 8/94	17.2	8.39	237
Upstream	Aug 27/94	18.1	8.49	227	Sep 9/94	14.6	8.63	216
Fort McMurray ds East	Aug 27/94	17.4	8.52	223	Sep 10/94	14	8.51	222
Mid-stream	Aug 27/94	17.4	8.57	235	Sep 10/94	13.2	8.59	214
West	Aug 27/94	17.2	8.54	222	Sep 10/94	13.5	8.55	215
Suncor	Aug 26/94	26.8	8.4	718	Sep 9/94	25.9	8.42	731
Downstream East	Aug 27/94	17.9	8.45	221	Sep 10/94	N/D	N/D	N/D
Mid-stream	Aug 27/94	17.9	8.48	225	Sep 10/94	13.9	8.6	217
West	Aug 27/94	17.9	8.45	218	Sep 10/94	N/D	N/D	N/D

N/D = not done

Table 5. Dates of deployment and recovery of SPMDs and numbers of SPMDs deployed and recovered at sites on Athabasca R., and % success of recovery of SPMDs.

Date deployed	Date removed	Site Location	# of SPMD's retrieved / # of SPMDs deployed
Aug 21	Sept 4	Hinton upstream	2/2
Aug 21	Sept 4	Hinton downstream	2/6
Aug 22	Sept 5	Weldwood mill in Hinton	3/3
Aug 22	Sept 5	Whitecourt upstream of Millar Western	2/2
Aug 22	Sept 5	Whitecourt downstream of Millar Western	2/6
Aug 23	Sept 6	Millar Western mill in Whitecourt	3/3
Aug 23	Sept 6	Alberta Newsprint mill, northeast of Whitecourt	3/3
Aug 23	Sept 6	Whitecourt upstream of Alberta Newsprint	0/2
Aug 23	Sept 6	Upstream of Slave Lake Pulp	2/2
Aug 24	Sept 7	Slave Lake Pulp mill	3/3
Aug 24	Sept 7	Downstream of Slave Lake Pulp	6/6
Aug 24	Sept 7	Athabasca R. below confluence of Lesser Slave R.	0/2
Aug 24	Sept 7	Upstream of Alberta-Pacific, at Athabasca	0/2
Aug 25	Sept 8	Alberta-Pacific mill, north of Boyle	0/3
Aug 25	Sept 8	Downstream of Alberta-Pacific, at Calling R.	4/6
Aug 26	Sept 9	Suncor plant, north of Fort McMurray	3/3
Aug 27	Sept 10	Downstream of Fort McMurray, at mile 11	2/2
Aug 27	Sept 10	Downstream of Oil Sands projects, at mile 33	6/6
Aug 27	Sept 10	Upstream of Fort McMurray	2/6
		Total # of SPMD's deployed	68
		Total # of SPMD's recovered	45
		% SPMD's recovered	66

Table 6. MFO activity (expressed as EROD potency equivalents, pg/g ± standard deviation) in PLHC-1 cells exposed to extracts of SPMDs that had been exposed to effluent or Athabasca or Lesser Slave River water for two weeks or exposed to air and handling contamination on deployment (trip blanks). Standard deviations were calculated from six replicate MFO dose response curves of one SPMD extract.

Location	Distance from Mill (km)	Trip Blank SPMD EROD potency-EQ (pg/g)	Exposed SPMD EROD potency-EQ (pg/g)
Hinton upstream, at Maskuta Creek	-7.0	10.3±0.7	14.0±1.1
Weldwood mill in Hinton	0.0	10.5±0.5	**62.0**±4.9
Hinton downstream, at Obed Mountain Bridge	36.0	3.9±0.2	20.8±1.2
Whitecourt, upstream of ANC, at Windfall Bridge	-20.0	7.2±0.5	lost R[a]
Alberta Newsprint mill, northeast of Whitecourt	0.0	6.9±0.4	**53.5**±3.2
Whitecourt, upstream of Millar Western	7.7/-2.0		lost A[b]
Millar Western mill in Whitecourt	0.0	23.9±1.7	**23.0**±1.4
Whitecourt downstream of M-W, at Blue Ridge	22.0	7.3±0.4	9.6±0.7
Lesser Slave R., upstream of Slave Lake Pulp	-2.0	9.5±0.7	16.9±0.8
Slave Lake Pulp mill	0.0	3.8±0.3	**29.7**±2.0
Lesser Slave R., downstream of Slave Lake Pulp	15.0	4.9±0.3	6.8±0.5, 5.1±0.4, 8.6±0.6
Athabasca R. below confluence of Lesser Slave R.	38.0		lost R
Athabasca R., upstream of Al-Pac, at Athabasca	-43.5		lost R
Alberta-Pacific mill, north of Boyle	0.0		**lost** R
Athabasca R.,downstream of Al-Pac, at Calling R.	28.5	3.0±0.2	10.8±0.8, 21.1±1.2
Upstream of Fort McMurray	-33.0	2.7±0.1	25.0±1.5
Downstream of Fort McMurray, at mile 11	-20.1	3.5±0.2	58.5±3.1, 728±89.7, 178±11.4
Suncor Inc., Oil Sands Group	0.0	4.0±0.2	**16800.0**±1470
Downstream of Oil Sands projects, at mile 33	15.3	2.7±0.2	309.0±31.8

lost R[a] = SPMD was lost in the river.
lost A[b] = SPMD was lost during the analysis and extraction phase

Legends to Figures

Figure 1. Map showing locations of SPMD deployment sites on the Athabasca River. SPMDs were deployed for 14 d during August and September, 1994, upstream and downstream (small circles) and in effluents of five pulp mills and one refinery. Numbers next to pulp mill symbols represent SPMD deployment sites in Weldwood of Canada, Ltd. (1), Alberta Newsprint Company, Ltd. (2), Millar Western Pulp, Ltd. (3), Slave Lake Pulp Corporation (4) and Alberta Pacific (5). SPMDs were also deployed in wastewaters of Suncor Inc., Oil Sands Group (6).

Figure 2. SPMD deployment device showing aluminum tube housing 2 to 3 SPMDs. Threaded rod was used to adjust the height of the SPMD from the patio stone anchor.

Figure 3. Water velocity (m/sec) measured at SPMD exposure sites on the Athabasca R. upon retrieval of SPMDs during September, 1994. Sites where one water velocity measurement (usually midstream) was taken are shown by white bars at each location. White, hatched and dark bars overlapping indicate readings taken at three sites across the river at that location. Points indicate replicate readings taken during SPMD deployment in August.

Figure 4. Temperature (°C) of Athabasca River water and effluents at SPMD deployment sites. Light bars show temperatures on deployment, late in August, 1994. Dark bars show temperatures two weeks later, in September, when SPMDs were removed.

Figure 5. pH of Athabasca River water and effluents at SPMD deployment sites. Light bars show pH reading taken on SPMD deployment, late in August, 1994. Dark bars show pH two weeks later, in September, when SPMDs were removed.

Figure 6. Conductivity (μS) of Athabasca River water and effluents at SPMD deployment sites. Light bars show conductivity taken on SPMD deployment, late in August, 1994. Dark bars show conductivity two weeks later, in September, when SPMDs were removed.

Figure 7. MFO activity in PLHC-1 cells caused by exposure to extracts of SPMDs from sites on Athabasca R and in effluents of four pulp mills or a refinery. Results are expressed as pg EROD potency-EQ/g SPMD. Extracts of SPMDs were compared to dose response curves of TCDD-exposed PLHC-1 cells, and potencies were determined by comparisons of EC50's.


ALBERTA
Slave River
Lake Athabasca
Peace River
Peace River
Peace River
Grande Prairie
Lesser Slave River
Wapiti River
Smoky River
Athabasca River
Fort McMurray
Edmonton
Calgary
1
2
3
4
5
6
SPMD Deployment Sites
Pulp Mills
Oil Sands Plant
River Deployment Sites

Figure 1.

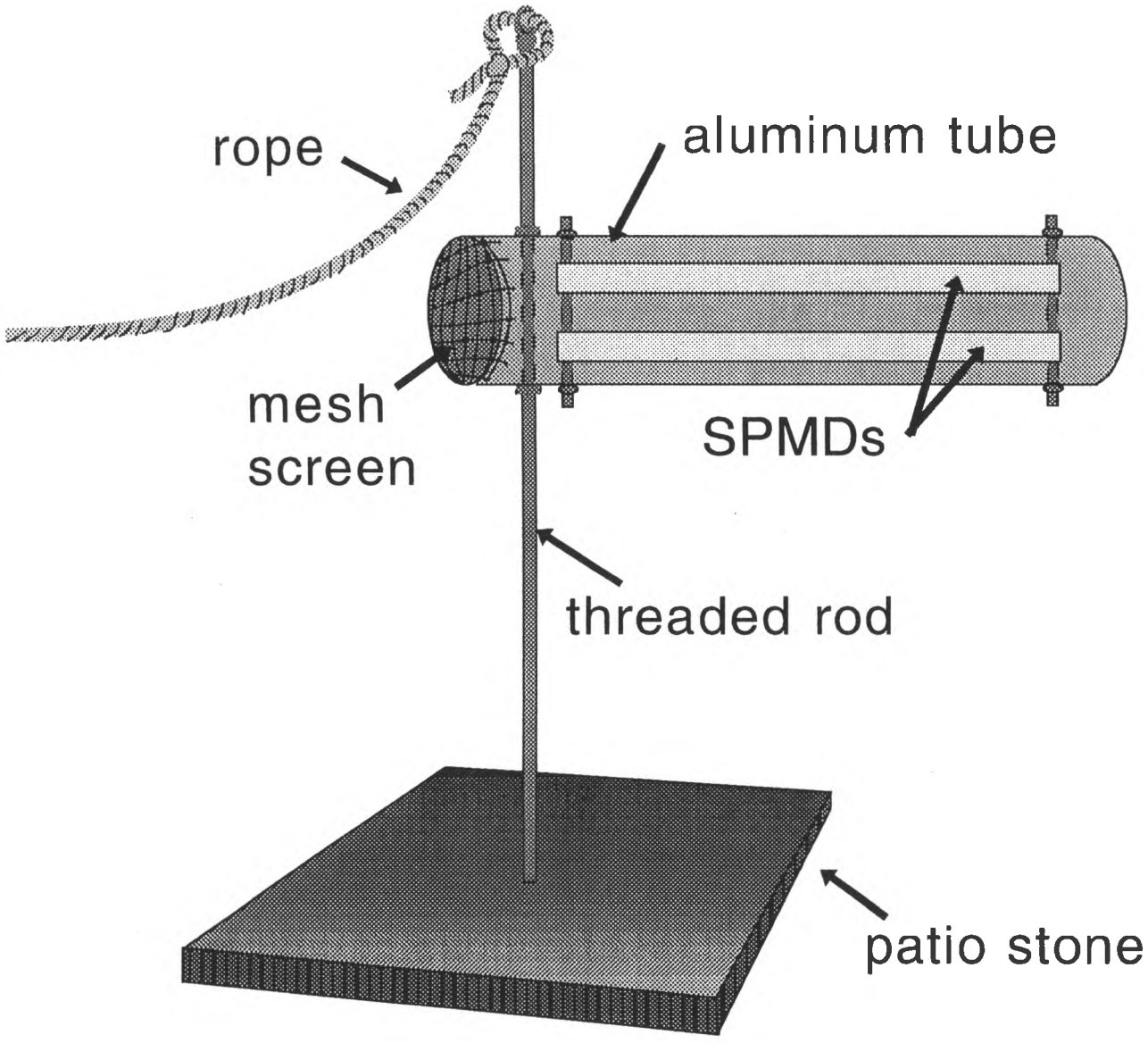
rope
aluminum tube
mesh
screen
SPMDs
threaded rod
patio stone

Figure 2.

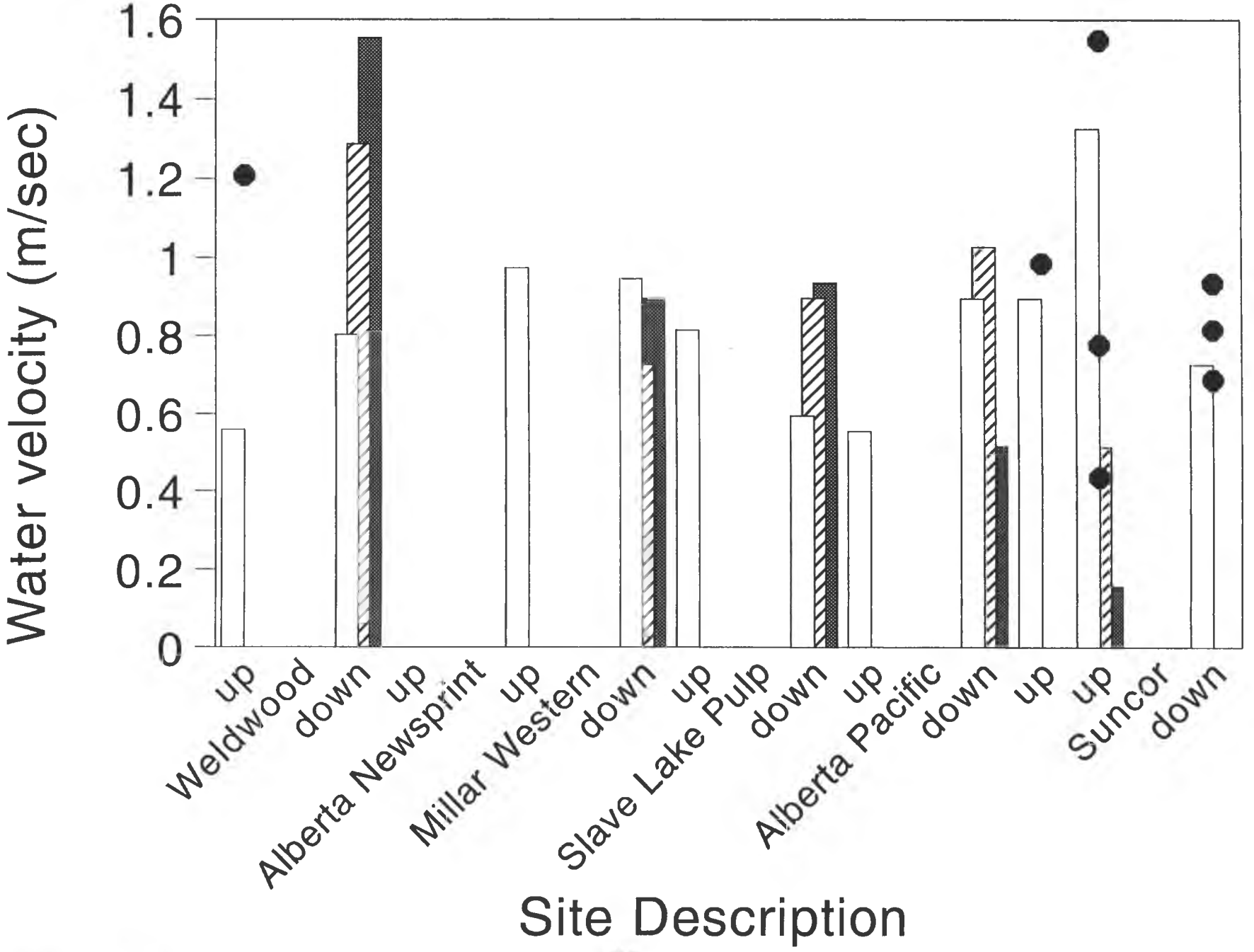
Water velocity (m/sec)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
up
Weldwood
down
up
Alberta Newsprint
up
Millar Western
down
up
Slave Lake Pulp
down
up
Alberta Pacific
down
up
up
Suncor
down
Site Description

Figure 3.

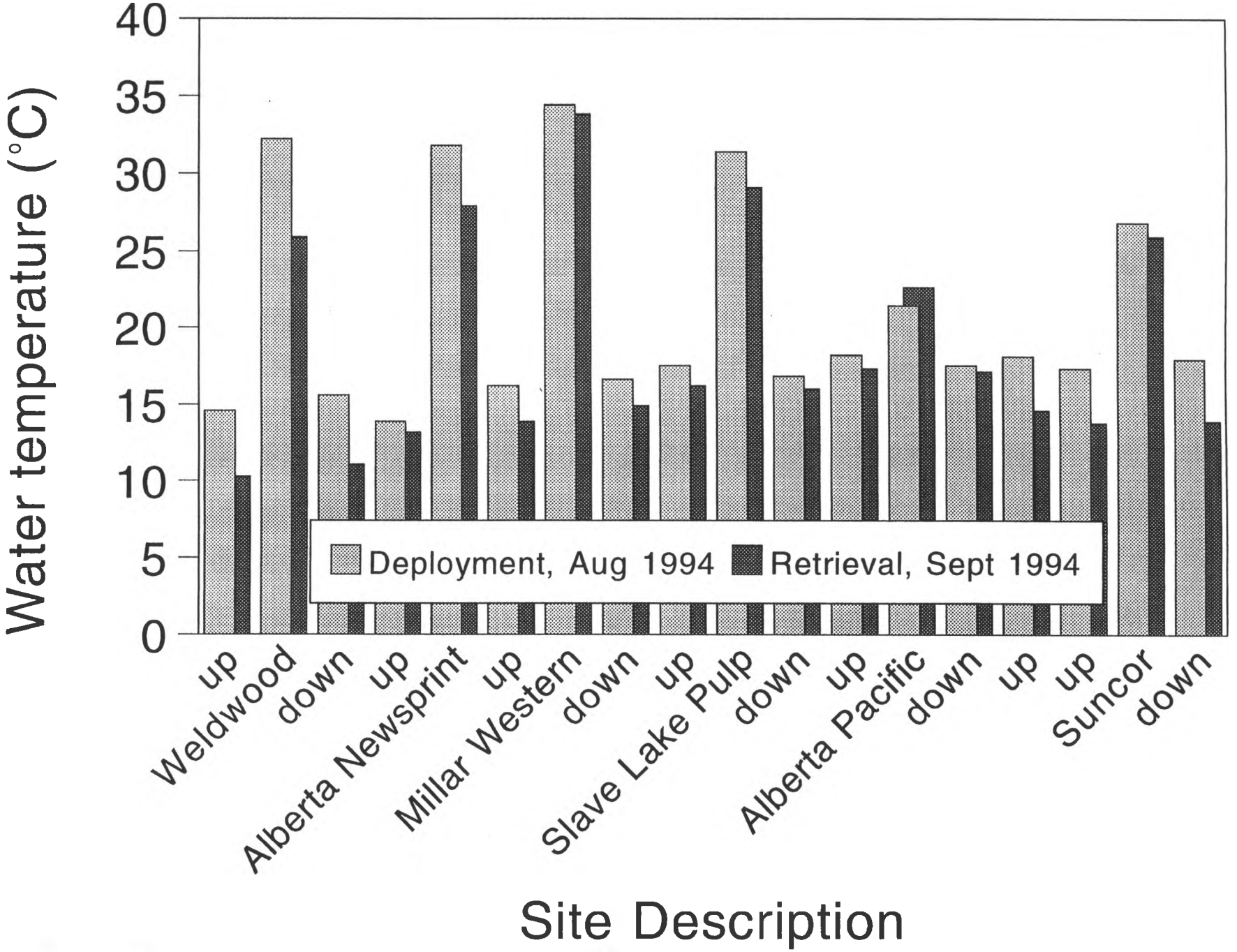
Water temperature (°C)
40
35
30
25
20
15
10
5
0
Deployment, Aug 1994
Retrieval, Sept 1994
up
Weldwood
down
up
Alberta Newsprint
up
Millar Western
down
up
Slave Lake Pulp
down
up
Alberta Pacific
down
up
up
Suncor
down
Site Description

Figure 4.

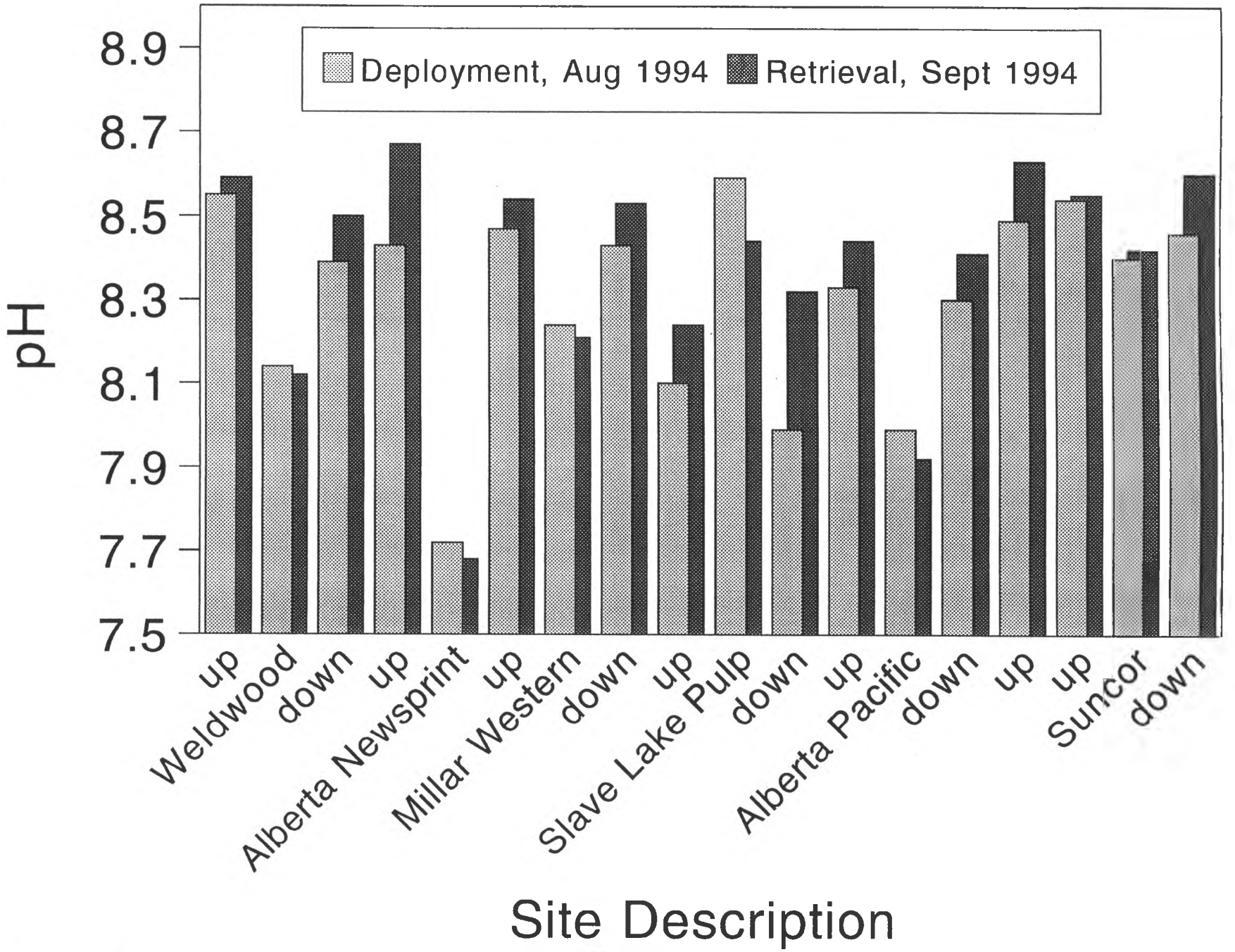
Deployment, Aug 1994
Retrieval, Sept 1994
pH
8.9
8.7
8.5
8.3
8.1
7.9
7.7
7.5
up
Weldwood
down
up
Alberta Newsprint
up
Millar Western
down
up
Slave Lake Pulp
down
up
Alberta Pacific
down
up
up
Suncor
down
Site Description

Figure 5.

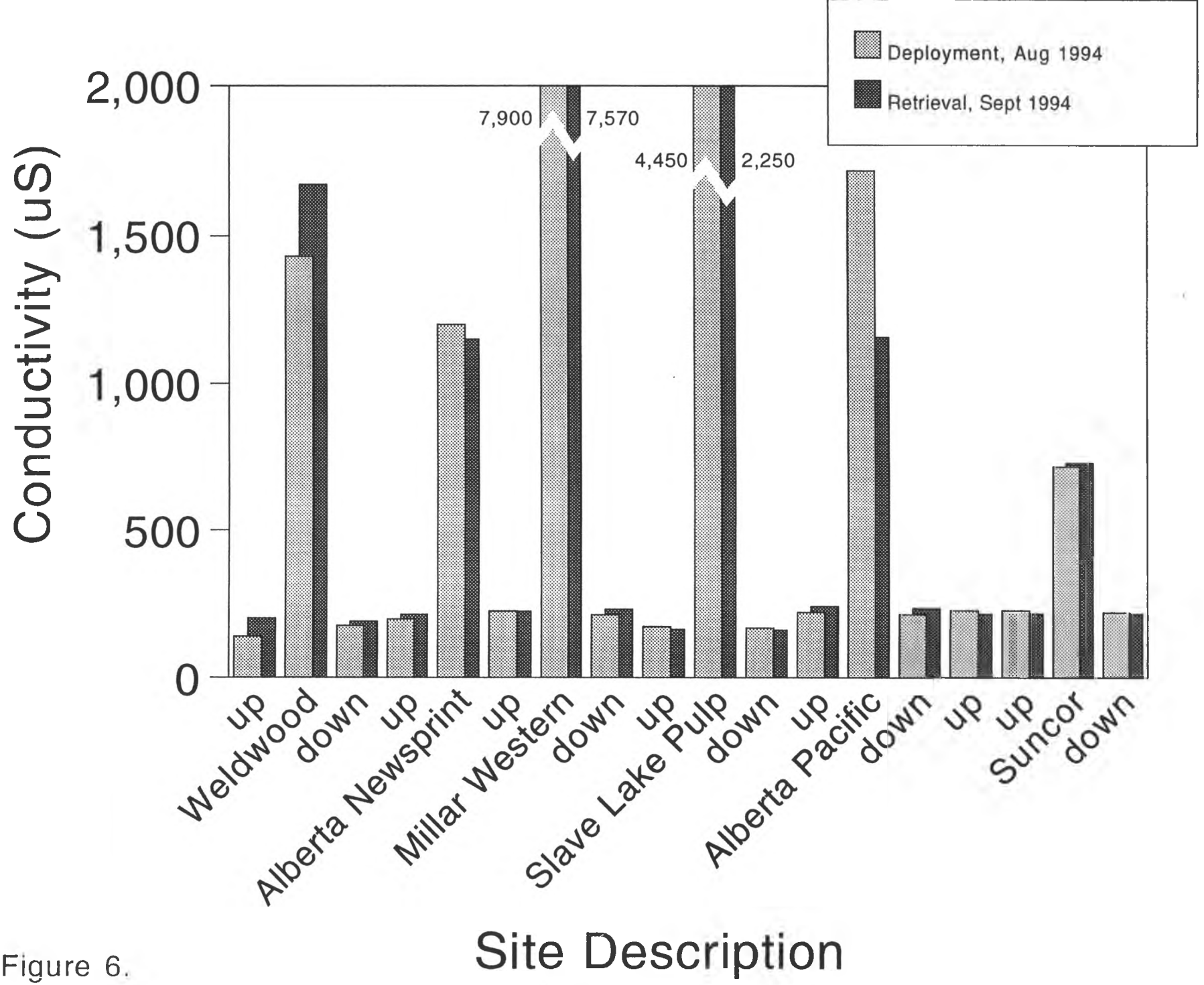
Deployment, Aug 1994
Retrieval, Sept 1994
Conductivity (uS)
2,000
1,500
1,000
500
0
7,900
7,570
4,450
2,250
up
Weldwood
down
up
Alberta Newsprint
up
Millar Western
down
up
Slave Lake Pulp
down
up
Alberta Pacific
down
up
up
Suncor
down
Site Description

Figure 6.

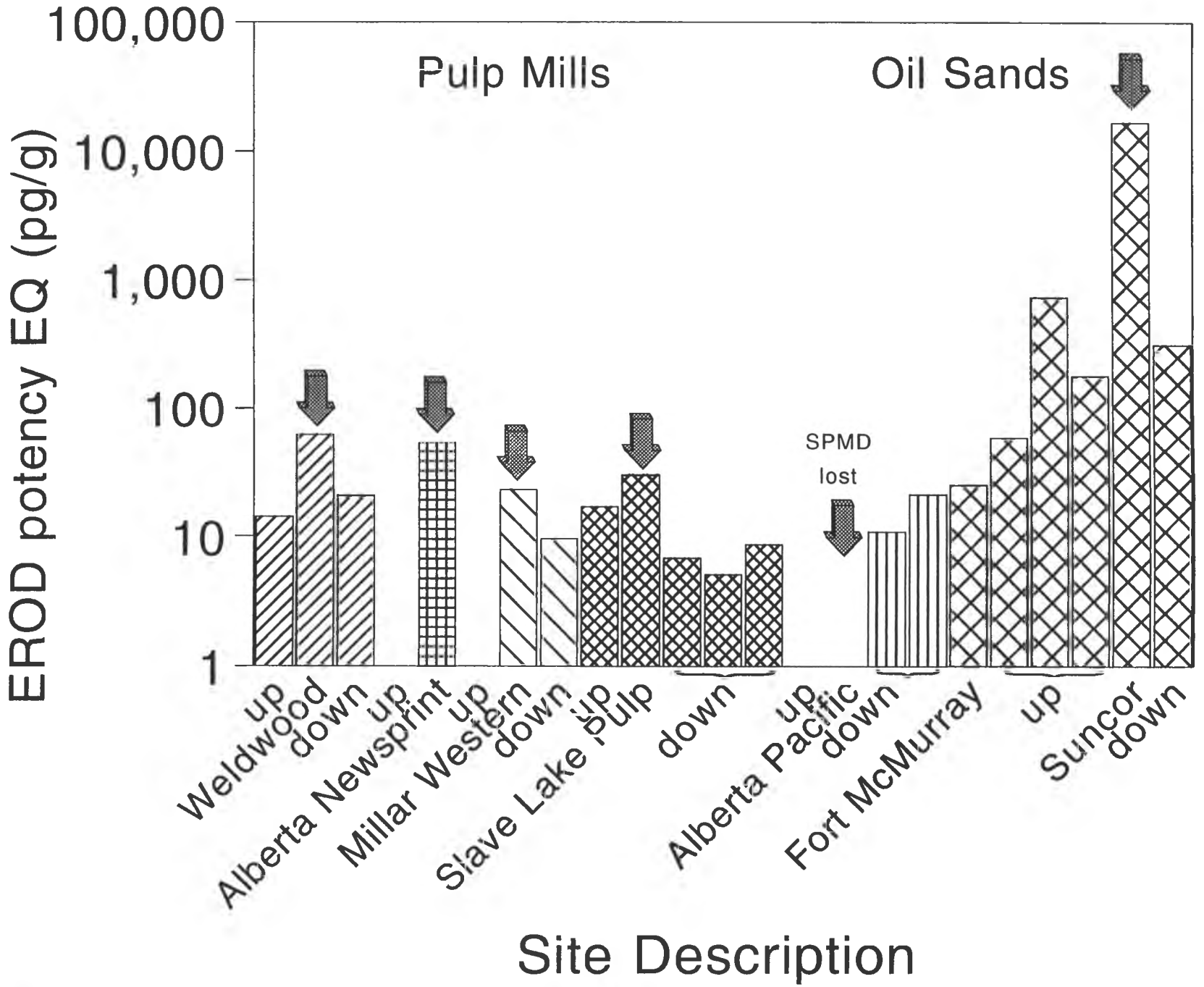
100,000
10,000
1,000
100
10
1
EROD potency EQ (pg/g)
Pulp Mills
Oil Sands
SPMD
lost
up
down
Weldwood
up
down
Alberta Newsprint
up
down
Millar Western
up
down
Slave Lake Pulp
up
down
Alberta Pacific
up
down
Fort McMurray
up
down
Suncor
Site Description

Figure 7.

APPENDIX A Terms of Reference

Project 2354-D1: Semi-Permeable Membrane Devices

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. Natural substrates for some MFOs include 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 includes various reactions which add molecular oxygen to lipophilic compounds. The terminal oxidase enzyme of the MFO system is the iron-containing hemoprotein cytochrome P-450. One group of cytochrome P-450s, called P-450IA (usually measured as ethoxyresorufin-O-deethylase (EROD) and arylhydrocarbon hydrolase (AHH) activity), is "induced" by the presence of several foreign compounds. That is, in the presence of these foreign compounds, animals synthesize new amounts of P-450IA proteins and enzyme activity is measurably increased. Induction is initiated when a foreign compound binds to the cellular Aryl hydrocarbon (Ah) receptor. Binding triggers the expression of the gene coding for P-450IA leading to increased RNA transcription and eventual synthesis of new P-450IA protein.

Experimental treatments with pure compounds have established that some polynuclear aromatic hydrocarbons and some chlorinated aromatic hydrocarbons induce, and turpenoid hydrocarbons possibly induce liver P-450IA in several fish species. The ability 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. 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 P-450IA 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 P-450IA 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 P-450IA 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. If induction is not detected, then presumably other Ah-associated biological responses are also not occurring. If induction is detected, more detailed studies are required on the bioaccumulation of inducers by local fish populations and on their survival, growth and reproduction. It must be recognized, however, that a lack of induction does not mean "no effect" - other effects may be produced by biochemical actions independent of the Ah receptor. Measurement of MFO induction signals only an increased probability of a suite of associated responses.

The purpose of this experiment is to identify pulp mill effluents along the Athabasca River system that induce MFO activity and to relate the extent of induction to variations in pulp mill processes.

MFO-inducing compounds will 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 (Huchins 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).

The primary advantage of SPMDs is one of logistics. SPMDs can be made to any size (usually, one meter long, 2-3 cm wide and 0.1 cm thick), can be rolled up and stored in a 200 mL sealed container, can be shipped by mail and can be deployed from shore, by wading or from any size boat. In laboratory studies, SPMDs have been found to accumulate inducers from pulp mill effluent, as shown by bioassays of extracts using fish cells in culture. 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 field collections of fish for MFO analyses, using caged fish *in-situ* experiments or exposing laboratory fish to stream water or to effluent shipped to the lab. One of the primary ecological advantages is that, through the use of SPMDs, spatial and temporal exposue to pulp mill effluents can be controlled.

II. Requirements

This contract will be carried out in five phases:

1. Phase I - The first phase will be preparative in nature. Personnel from regional offices of Environment Canada and Alberta Environmental Protection will be contacted to solicit their cooperation and assistance with this study and to seek their opinions as to the most appropriate sites for the deployment of SPMDs. Contacts will also be made with representatives from industries in the Athabasca River system to inform them of this study.

2. Phase II - The second phase will be the preparation and packaging of SPMDs, with appropriate documentation describing where and how they will be deployed, and the construction of equipment for deployment.

3. Phase III - The third phase will involve the deployment of the SPMDs at sites along the Athabasca River between August 15th and September 30, 1994. Ideally, the sampling should occur during a declining hydrograph and at temperatures between 15 and 20°C. Although flows in the Athabasca River are declining during this time period, the water temperatures will be slightly lower (i.e., between 12 and 15°C) than ideal conditions.

    A. SPMDs are to be prepared and deployed in duplicate to sample compounds that cause MFO induction in the effluent from each of the pulp mills and the receiving water upstream of the mixing zone (reference) of each of the mills. 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.

Up to sixty SPMDs are to be deployed. Specifically, the SPMDs are to be deployed at the following locations:

i) upstream of the Hinton Combined Effluent (2);
ii) in the Weldwood or Hinton Combined Effluent (2);
iii) downstream of Hinton (6);
iv) upstream of Whitecourt (2);
v) in the Alberta Newsprint effluent discharge (2);
vi) in the Millar Western effluent discharge (2);
vii) downstream of Whitecourt (6);
viii) above the confluence of the Athabasca and Lesser Slave rivers (2);
ix) upstream of Alpac (2);
x) in the Alpac effluent discharge (2);
xi) downstream of Alpac (6);
xii) upstream of Slave Lake pulp (2);
xiii) in the effluent discharge of Slave Lake Pulp (2);
xiv) downstream of Slave Lake Pulp on the Lesser Slave River (6);
xv) upstream of Fort McMurray (2);
xvi) downstream of Fort McMurray (6);
xvii) in the Suncor effluent (2); and,
xvii) downstream of the tar sands (6).
( ) indicates the number of SPMDs to be employed at each sites.

A total of 36 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 Athabasca River, 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 in dry ice or liquid nitrogen for shipment. All samples must be maintained at -20°C during shipping.

4. Phase IV - The SPMDs will be returned to the lab, extracted into solvent and split for analysis (see Huchins et al. 1994). The samples will be tested for MFO inducing ability with cell culture techniques using fish cell line assays.

Potency (i.e., amount of inducer) will be expressed in 2,3,7,8 - TCDD toxic equivalency (TEQ) based on cell lines exposed to standard levels of dioxins.

5. Phase V - The data will be compiled and comparisons of induction made to mill characteristics and the distance from the effluent discharges.

III. Deliverables

Following analyses of the data, a summary report and an electronic disk copy of the data are to be submitted by March 31, 1995. A Draft report is to be submitted by June 1, 1995, along with six to ten 35 mm slides that can be used at public meetings to summarize the project, methods and key findings.

IV. Reporting Requirements

1. Ten copies of the Draft Report along with an electronic disk copy are to be submitted to the Project Liaison Officer by June 1, 1995.

2. Three weeks after the receipt of review comments on the draft report, the Contractor is to provide the Project Liaison Officer 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 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.

   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 Project Liaison Officer 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.

4. All figures and maps are to be delivered in both hard copy (paper) and digital formats. Acceptable formats include: DXF, uncompressed E00, VEC/VEH, Atlas and ISIF. All digital maps must be properly geo-referenced.

5. 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).

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

V. Contract Administration

The Scientific Authority for this project is:

Dr. Peter Hodson
National Water Research Institute
867 Lakeshore Road
P.O. Box 5050
Burlington, Ontario
L7R 4A6
phone: (905) 336-4778
fax: (905) 336-6430

Questions of a scientific nature should be directed to him.

The NRBS Study Office Component Coordinator for this project is:

Rick 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

Huchins, 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.