INITIAL EEM FISH COLLECTION AND TISSUE ANALYSES, ATHABASCA RIVER NEAR WHITECOURT, ALBERTA

MAY 1993

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ATHABASCA RIVER NEAR WHITECOURT, ALBERTA

Prepared for:

Alberta Newsprint Company
Whitecourt, Alberta

Prepared by:

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Calgary, Alberta

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SENTAR

May 26, 1993
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Mr. Brian Steinback
Alberta Newsprint Company
Postal Bag 9000
Whitecourt, Alberta
T0E 2L0

Dear Mr. Steinback:

Reference: Initial EEM Fish Collection and Tissue Analysis

Please find attached our final report on the above mentioned study. We have incorporated the results of the forage fish collection study along with your comments on an earlier draft.

We hope this document provides some guidance into your EEM planning.

Yours truly,

SENTAR CONSULTANTS LTD.

Bob Shelast, P. Biol.
Senior Aquatic Biologist

BS/bs

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

1.0 INTRODUCTION

Alberta Newsprint Company (ANC) operates an integrated thermomechanical pulp (TMP) and paper mill near Whitecourt, Alberta. The pulp and paper mill became operational in August, 1990. Water is obtained from the Athabasca River for process use and following treatment, effluent is discharged to the Athabasca River at a rate of about 15,000 m^3/day. In May 1992, the Pulp and Paper Regulations under the Fisheries Act were amended to include the requirement for Environmental Effects Monitoring (EEM) studies for the pulp and paper industry. The purpose of EEM is to monitor the effects of pulp and paper effluents in the aquatic environment.

As preparation for EEM, ANC decided to conduct a preliminary assessment of fish health and contaminant levels in fish tissue in a near-field area downstream of their effluent discharge. SENTAR Consultants Ltd. was retained to conduct the initial fish collection and tissue analysis for this study. The objective of the study was to determine contaminant levels and fish health in fish that overwinter below, and are close proximity to, the ANC effluent outfall. It was assumed that these fish would be relatively sedentary during the winter months and would, therefore, be exposed to effluent discharge over a substantial period of time. During open-water seasons, some fish species are very mobile, which raises uncertainty regarding time of exposure. The information collected during this study would assist in planning the pre-design and core study of EEM.

2.0 METHODS

Previous fisheries studies on the river indicated that suitable overwintering habitat was present at a site immediately upstream of the ANC railway bridge, which is about 1 km upstream of the Highway #43 bridge crossing (EMA 1993). This site is about 7 km below the ANC effluent diffuser but above Millar Western's effluent outfall. Two species of fish, mountain whitefish (*Prosopium williamsoni*) and longnose suckers (*Catostomus catostomus*), were selected as target species since they were the only species which were abundant enough to offer a reasonable chance of obtaining a large enough sample size.

Fish collections were made on March 10 and 11, 1993 by angling through the ice at a site under the railway bridge (Plate 1). Twelve to eighteen lines with baited (dew worms, maggots) lures were fished. Ice depth at the site averaged 1 m and water depth was 1.8 m. This site was characterized by a relatively low current velocity with a cobble substrate. Angling was attempted in the main channel where water depth was >3 m; however, large amounts of frazil ice precluded effective angling. Flows in the Athabasca River at Windfall during the sampling period ranged from 41.6 to 53.5 m^3/s (Alberta Environmental Protection, River Forecast Centre, pers. comm.)

Blood was withdrawn from fish within 15 minutes of capture following the Standard Operating Protocols developed by SENTAR (Plate 2). Fish were then sacrificed and weighed, length measured and examined for external pathology. Fish were then dissected, examined for internal pathology and tissue (muscle, liver, gonad, heart, spleen, kidney, gill, stomach/intestine) and bile samples taken following SENTAR's Standard Operating Protocols (Plates 3 and 4). Depending on the type of tissue, samples were preserved in either liquid nitrogen, on dry ice or in buffered formalin. Appropriate ageing structures were taken from each fish.

Chemical analyses for dioxin and furan congeners in muscle and liver tissue, resin and fatty acids in bile, and ethoxyresorufin-O-deethylase (EROD) activity were done by EnviroTest Laboratories in Edmonton Routine clinical measurements in blood serum were done by Veterinary Pathology Laboratory (VPL) in Edmonton. GlobalTox of Ottawa was responsible for the histopathological assessment of tissue samples.

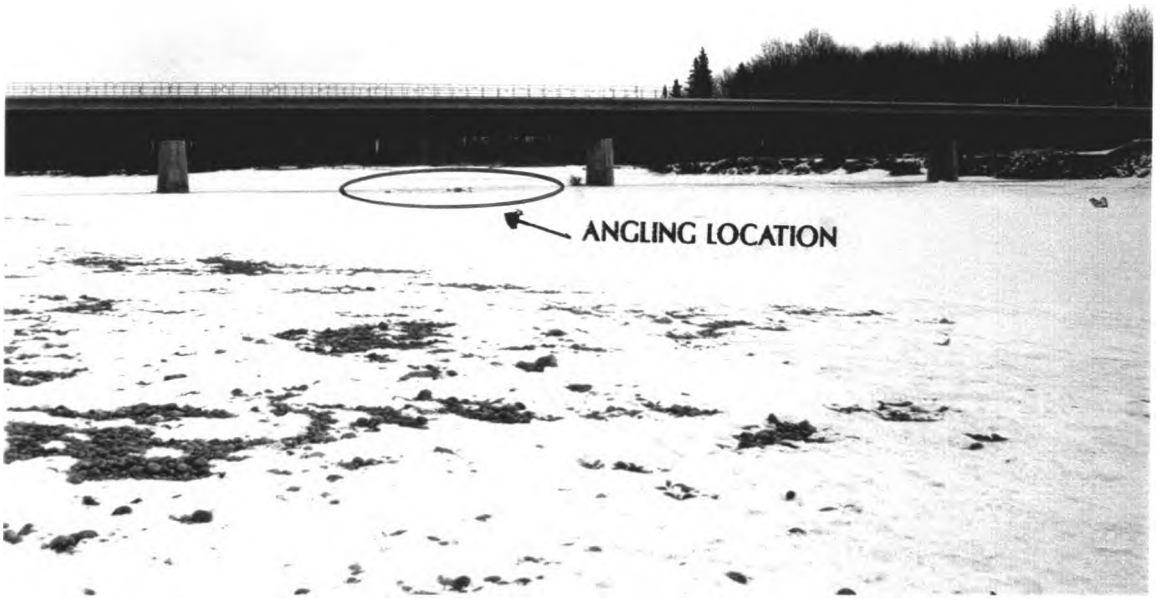

Plate 1. Sampling location.

Plate 2. Drawing blood sample.

Plate 3. Dissecting mountain whitefish for tissue sampling.

Plate 4. Gall bladder filled with bile.

3.0 RESULTS AND DISCUSSION

Three mountain whitefish were the only fish species captured during the study. These fish ranged in size from 329 to 435 mm and weighed between 485 and 1095 g (Table 1). Age classes 5, 7 and 9 were represented by the sample. Two of the fish were resting males (i.e. gonads wil mature in the next spawning season) while the other was a green female (has not previously spawned).

3.1 BLOOD CHEMISTRY

Blood chemistry analysis indicated that there was slight variation in blood chemistry measurements among the three mountain whitefish sampled (Table 2). Although "normal" values are not known for this species, Folmar (1993) presents a range of values for other salmonids which can be used for comparative purposes.

Electrolyte (sodium, potassium, chloride, calcium, phosphorus) values were within the normal range for salmonids with the exception of potassium. Electrolyte values can differ seasonally (e.g. calcium, potassium) and by sex (e.g. calcium). Although serum potassium levels between 2 to 12 meq/L have been reported in salmonids of the genus *Oncorhynchus* (Folmar 1993), the concentrations found in this study (9.1 to 10.2 meq/L) are at the upper limit of normal values. These elevated levels of potassium may be indicative of capture stress and represent a response to handling since potassium can be released into the blood stream very rapidly.

Of all the blood chemistry measurements, the plasma enzymes have the greatest variability (Folmar 1993). In this study, alanine aminotransferase (ALT) levels ranged from 13 to 37 IU/L and did not appear to be related to age or size of fish. Folmar (1993) reported that in brown trout, ALT levels were quite variable with mean values ranging from 4.8 to 42 IU/L. Most studies of rainbow trout reported ALT values of 4 to 13 IU/L: however, one study reported values as high as 136 IU/L for this species. Alkaline phosphatase (ALP) levels in mountain whitefish from this study ranged from 212 to 313 IU/L. This enzyme is known to vary seasonally in adult Atlantic salmon. ALP activity appeared to be size related in rainbow trout with larger fish showing an order of magnitude greater activity than juvenile fish (100-300 IU/L cf. 9-12 IU/L). Amylase, an enzyme which hydrolyzes starch, ranged between 318 and 457 IU/L in mountain whitefish from the Athabasca River while lipase,

Table 1. Summary of biological characteristics of mountain whitefish taken in the Athabasca River near Whitecourt, March 1993.

Fish Number	Length (mm)	Weight (g)	Age	Sex	Maturity	Condition
MTWT 001	329	485	5	F	Green	1.36
MTWT 002	417	1050	9	M	Resting	1.45
MTWT 003	435	1095	7	M	Resting	1.33

Table 2. Values for routine clinical measurements in blood serum from mountain whitefish, Athabasca River near Whitecourt, March 1993.

Parameter	Sample Number		
	MTWT 001	MTWT002	MTWT003
Sodium (meq/L)	148	145	148
Potassium (meq/L)	10.2	9.1	9.3
Chloride (meq/L)	134	130	134
Calcium (meq/L)	3.29	3.47	3.23
Phosphorus (meq/L)	3.33	4.05	3.91
Carbon Dioxide (meq/L)	14	13	13
Alanine Aminotransferase (IU/L)	31	37	13
Alkaline Phosphatase (IU/L)	239	313	212
Amylase (IU/L)	318	457	381
Lipase (IU/L)	110	130	118
Total Bilirubin (µmol/L)	2	12	14
Blood Urea Nitrogen (meq/L)	<0.4	<0.4	<0.4
Glucose (meq/L)	7.8	8.9	8.3
Total Protein (g/L)	38	40	41
Albumin (g/L)	17	18	19
Globulin (g/L)	21	22	22
Creatinine (µmol/L)	18	20	19
Methemoglobin (%)	0.12	0.11	0.10
Hemoglobin (%)	14.0	17.3	17.2
Hematocrit (%)	50	62	55

an enzyme that hydrolyzes fats, ranged between 110 and 130 IU/L. Normal levels of these enzymes in salmonids are not available.

Total protein in the three mountain whitefish captured in this study was similar ranging between 38 and 41 g/L. Folmar (1993) reports total protein concentrations in rainbow trout, brown trout and brook trout ranging from 20 to 100, 41 to 57 and 40 to 70 g/L, respectively. Serum protein concentrations in these species exhibited distinct seasonal and sex related differences. Exposure to pulp mill effluent resulted in a decrease in serum total protein. Albumin and globulin, two specific blood proteins, were in similar concentrations in the three mountain whitefish studied.

Hematocrits in Athabasca River mountain whitefish ranged from 50 to 62 % which is above the range reported for adult Atlantic salmon (44 - 49 %), rainbow trout (32 - 59 %), brook trout (30 - 48 %) and brown trout (20 - 56 %). Folmar (1993) reported that exposure of rainbow trout to unbleached kraft mill effluent resulted in a decreased hematocrit.

3.2 HISTOPATHOLOGY

Histopathological examination of the three mountain whitefish indicated no significant abnormalities (Appendix 1). Fish MTWT 002 was diagnosed as having severe multifocal splenic hemosiderosis, meaning that iron metabolism, such as excessive breakdown of hemoglobin, had occurred in the past. This condition was not likely to have caused problems for the fish and may have resulted from an old hemorrhage noted between the pectoral and pelvic fins. Fish MTWT 003 was diagnosed with multiple splenic lipomata which are benign tumours of little significance to the fish. Based on the histopathological diagnosis, all three mountain whitefish would be considered normal.

Implications of Blood Chemistry and Histopathology Data for ANC's Future Monitoring Plans

The blood chemistry and histopathology data do not present the "pulp mill suite" of symptoms found in other studies. However, small sample size and the effects of capture stress prevent any firm conclusions at this time. We recommend that both blood chemistry and histopathology be included in future monitoring because these data will provide additional "weight of evidence" regarding effects on fish health in what is a complex situation.

3.3 DIOXAN/FURAN AND RESIN/FATTY ACIDS ANALYSES

All three mountain whitefish analysed contained residues of 2,3,7,8-TCDD, 2,3,7,8-TCDF and 2,3,8-TriCDF in both muscle and liver tissue (Table 3 and Appendix 2). No other congeners were found.

The 2,3,7,8-TCDD/TCDF residues are lower than those recorded in mountain whitefish downstream of Hinton in 1988 (Environment Canada, Fisheries and Oceans Canada, Health and Welfare Canada 1988). The fish analysed downstream of Hinton contained 4 to 32 ppt 2,3,7,8-TCDD and 8 to 58 ppt 2,3,7,8-TCDF. Health and Welfare Canada suggest a guideline limit of 2,3,7,8-TCDD in fish muscle of 20 ppt for human consumption. Other congeners were generally absent in Hinton fish, with one exception; one mountain whitefish contained some hexadioxin.

The residues found in the mountain whitefish downstream of ANC are typical of those associated with bleached kraft pulp mills. Given the existing evidence for accumulation of 2,3,7,8-TCDD/TCDF in fish downstream of Hinton, it is likely that the results from the ANC fish reflect bleached kraft effluent input upstream.

Resin and fatty acids were not detected in the bile of the mountain whitefish taken below ANC (Appendix 2).

Implications of Dioxin/Furan Data for ANC's Future Monitoring Plans

It is encouraging that the more highly-substituted congeners that would be indicative of a recycled-newsprint source were not found in the three mountain whitefish. Larger sample sizes are required to confirm the lack of uptake; however, given that the three fish were exposed to low-flow, low-dilution conditions (0.4 - 0.5 %), the outlook for minimal to no uptake appears promising.

The presence of kraft mill-related congeners in the fish clearly indicates the mobility of mountain whitefish and the long biological half-time of 2,3,7,8-TCDD/TCDF in this species. Because of the persistent residues from another source, potential health effects on this species will have to be investigated with great care. Suggestions for some approaches to this problem are presented in the EROD section.

Table 3. Summary of dioxin and furan analyses (pg/g) for mountain whitefish, Athabasca River near Whitecourt, March 1993.

Congener	Sample Number							
	MTWT 001		MTWT 002		MTWT 003		Detection Limit	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
2,7/2,8-DiCDD	ND	ND	ND	ND	ND	ND	0.2-0.4	0.1-2.8
2,3-DiCDD	ND	ND	ND	ND	ND	ND	0.1-0.4	0.2-2.4
2,3,7-TriCDD	ND	ND	ND	ND	ND	ND	0.1-0.2	0.1-2.3
2,3,7,8-TCDD	1.0	1.6	0.8	3.6	6.3	0.7	0.1-0.2	0.7-2.5
1,2,3,7,8-PeCDD	ND	ND	ND	ND	ND	ND	0.1-0.2	1.3-150
1,2,3,4,7,8-HxCDD	ND	ND	ND	ND	ND	ND	0.2-0.5	1.0-2.6
1,2,3,6,7,8-HxCDD	ND	ND	ND	ND	ND	ND	0.2-0.6	1.4-9.0
1,2,3,7,8,9-HxCDD	ND	ND	ND	ND	ND	ND	0.2-0.6	1.5-2.6
1,2,3,4,6,7,8-HpCDD	ND	ND	ND	ND	ND	ND	0.3-0.8	1.4-24
OCDD	ND	ND	ND	ND	ND	ND	0.9-2.8	8.3-44
2,8-DiCDF	ND	ND	ND	ND	ND	ND	0.1-0.3	0.4-3.1
2,3,8-TriCDF	1.5	4.0	1.9	2.2	1.9	3.0	0.1-0.2	0.2-1.2
2,3,7,8-TCDF	3.6	3.1	4.0	6.9	19.0	3.0	0.1-0.2	0.7-2.3
1,2,3,7,8-PeCDF	ND	ND	ND	ND	ND	ND	0.1-0.2	1.4-47
2,3,4,7,8-PeCDF	ND	ND	ND	ND	ND	ND	0.1-0.2	1.3-100
1,2,3,4,7,8-HxCDF	ND	ND	ND	ND	ND	ND	0.2-0.4	1.3-5.7
1,2,3,6,7,8-HxCDF	ND	ND	ND	ND	ND	ND	0.2-0.4	1.2-5.5
2,3,4,6,7,8-HxCDF	ND	ND	ND	ND	ND	ND	0.2-0.4	1.4-7.9
1,2,3,7,8,9-HxCDF	ND	ND	ND	ND	ND	ND	0.3-0.6	2.1-17
1,2,3,4,6,7,8-HpCDF	ND	ND	ND	ND	ND	ND	0.2-0.4	1.5-12
1,2,3,4,7,8,9-HpCDF	ND	ND	ND	ND	ND	ND	0.4-0.9	2.6-26
OCDF	ND	ND	ND	ND	ND	ND	0.7-2.2	13-80

ND - Not Detected

3.4 HEPATIC EROD ACTIVITY IN MOUNTAIN WHITEFISH

The activity of ethoxyresorufin-O-deethylase (EROD) is used as an indicator of the activation of liver detoxification systems. These systems respond to exposure to a variety of chemicals. The system that EROD represents is known to respond to dioxins/furan, polychlorinated biphenyls (PCB's), and polycyclic aromatic hydrocarbons(PAH's) (Stegeman 1981).

The three mountain whitefish from ANC had EROD activities that were "moderately induced". Activities were higher than those found in the North Saskatchewan River upstream of Rocky Mountain House, which receives no effluent inputs(Table 4). However, they were lower than activities in fish from the Wapiti/Smoky River downstream of the Weyerhaeuser mill at Grande Prairie (Table 4) (Swanson et al. 1993).

The cause of the moderate EROD induction may be the dioxin/furan exposure indicated by the body burdens of 2,3,7,8-TCDD/TCDF found in these fish. However, EROD induction downstream of pulp mills may be multi-factorial. For example, EROD induction quickly disappeared at an Ontario pulp mill site when effluent discharge ceased during a shut-down (Munkittrick et al. 1992). If dioxin/furan body burden was the cause of the induction, one would have expected the induction to persist. A survey of 12 pulp mills in Ontario has shown EROD induction at all mill sites, irrespective of mill type or effluent treatment (some were bleached kraft, some were unbleached kraft and some were sulfite) (Carey et al. 1993). Government scientists now theorize that there may be compounds produced in the pulping process (prior to any bleaching) which may induce EROD activity. These compounds appear to have transitory effects (since induction disappears during shut-downs).

In contrast to the Ontario mill data, fish from the Wapiti/Smoky RIver continued to be induced even when removed to cages upstream of the effluent. A correlation with dioxin/furan body burden was noted in 1991. In this case, induction was not transitory. Studies now underway after 100% chlorine dioxide substitution may shed more light on the causes of induction in Wapiti/Smoky fish.

Irrespective of the exact causes of EROD induction, evidence is mounting that it is commonly found downstream of pulp mills. The question then is "so what?". There is no compelling evidence in the literature that EROD induction is correlated with adverse effects

Table 4. **Hepatic EROD specific activity in mountain whitefish from the Athabasca, Wapiti/Smoky and North Saskatchewan rivers.**

Location	Season/Year	Hepatic EROD Specific Activity (nmol/min/mg)			n
		Mean ± Standard Deviation	Minimum	Maximum	
Athabasca River	March 1993	0.109 ± 0.012	0.097	0.120	3
Wapiti/Smoky River	Spring 1991	0.557 ± 0.420	0.024	1.747	18
	Fall 1991	0.227 ± 0.112	0.038	0.408	10
	Spring 1992	0.218 ± 0.125	0.054	0.631	19
North Saskatchewan River	Spring 1991	0.021 ± 0.014	0.003	0.060	24
	Fall 1991	0.029 ± 0.013	0.015	0.042	6

on fish health. The Ontario 12-mill study apparently shows reduced sex steroids at most of the sites; however, the actual data have yet to complete peer-review. Therefore, at present, EROD induction can be taken as an indication of exposure to pulp mill effluent, but it is not an indication of effects.

Implications of EROD Data for ANC's Future Monitoring Plans

There are two questions about the apparently "induced" fish observed downstream of ANC:

(1) what is the cause of the induction?

(2) are there associated effects on fish health?

The first question involves sorting out whether the induction is due to persistent compounds present in bleached kraft effluent from upstream or whether induction is in response to ANC's effluent. Cage studies may be one approach to answering this question. In cage studies, fish from a pulp mill-free stream would be placed both upstream and downstream of ANC and the degree of induction compared. The cage studies would have to be designed to ensure that fish are exposed to the ambient substrate (hence, food supply) and water column.

Because studies of EROD induction are not a requirement of EEM, proceeding with such work would be in response to more general concerns arising out of provincial regulatory agencies and out of any data being generated by the Northern River Basins Study (NRBS). Furthermore, although EEM requirements do not include EROD, such measurements are strongly recommended.

The question of effects on fish health will probably centre on sex steroid effects because these are the effects observed at Ontario mills. Again, EEM requirements do not call for such work; however, NRBS is including sex steroids in its measurements of Athabasca fish. Therefore, it may be advisable to include some industry work on this subject. Because of the complexity of this type of monitoring, cooperation among several mills may be the most practical approach.

A cooperative study with other pulp mills of sex steroid effects would involve collection of estradiol, testosterone, 17,20-dihydroprogesterone and 11-keto testosterone data from at least one major study species at several locations in the Athabasca River and from at least one reference area. Data should be collected during both the inter-spawning and

spawning periods. Sample sizes should be as large as practical, since one of the limitations of the work done by government scientists to date is small sample size and highly-variable data.

Sex steroid studies must be accompanied by concurrent studies of reproductive capacity in populations. These studies would include measurement of fecundity, age-to-maturity, egg size and recruitment of young-of-the-year. Without these studies, the relevance of hormone data will be unknown (e.g. Ontario mill data show effects on sex steroids but not effects on overall population reproduction). These data will be collected during the course of EEM Adult Fish Surveys; however, care would have to be taken that the same species used in the cooperative steroid study are included in EEM work.

3.5 CHLORINATED PHENOLICS

The types of chlorinated phenolics detected in the bile (Table 5) are similar to those found in mountain whitefish in the Wapiti River downstream of the Grande Prairie mill (Swanson et al. 1993) and are typical of those associated with kraft mill effluent. For example, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 4,5-dichloroguaiacol, 3,4,5-trichloroguaiacol and tetrachloroguaiacol are found in kraft mill effluent and were detected in both Wapiti River fish and the fish in this study.

Concentration of chlorinated phenolic compounds in the fish in this study were somewhat lower than those found in Wapiti River fish. This is not unexpected since the Wapiti data are primarily from fish caught close to the effluent discharge. However, concentrations in the fish from the Smoky River, 230 km downstream of the Grande Prairie mill were quite similar to those in this study. Thus, the data from the fish downstream of ANC are consistent with a far-upstream kraft pulp mill source.

3.6 OVERALL IMPLICATIONS FOR THE EEM ADULT FISH SURVEY

The data clearly indicate that confounding effects from upstream discharges will have to be considered. We suggest that ANC consider a two-pronged approach:

(1) monitor mountain whitefish with an experimental approach utilizing cage studies and perhaps a cooperative sex steroid study;

(2) monitor a fish species more likely to be resident (such as the forage species longnose dace).

Table 5. Summary of chlorinated phenolics analyses (ppm) in mountain whitefish bile, Athabasca River near Whitecourt, March 1993.

Compound[a]	Sample Number		
	MTWT 001	MTWT002	MTWT003
2-Chlorophenol	ND	ND	ND
4-Chlorophenol	ND	ND	ND
2,4-Dichlorophenol	0.58	0.39	0.59
2,6-Dichlorophenol	ND	ND	ND
2,4,5-Trichlorophenol	ND	ND	ND
2,4,6-Trichlorophenol	1.70	1.10	1.30
2,3,4,6-Tetrachlorophenol	0.19	ND	ND
Pentachlorophenol	ND	ND	ND
4-Chlorocatechol	ND	ND	ND
3,4-Dichlorocatechol	ND	ND	ND
3,6-Dichlorocatechol	ND	ND	ND
3,5-Dichlorocatechol	ND	ND	ND
4,5-Dichlorocatechol	ND	ND	ND
3,4,5-Trichlorocatechol	ND	ND	ND
3,4,6-Trichlorocatechol	ND	ND	ND
Tetrachlorocatechol	ND	ND	ND
4-Chloroguaiacol	ND	ND	ND
3,4-Dichloroguaiacol	1.30	0.88	1.50
4,5-Dichloroguaiacol	0.49	0.29	.049
4,6-Dichloroguaiacol	ND	ND	ND
3,4,5-Trichloroguaiacol	6.50	4.20	6.60
3,4,6-Trichloroguaiacol	0.19	ND	0.20
4,5,6-Trichloroguaiacol	1.40	0.88	1.20
Tetrachloroguaiacol	4.10	2.50	3.30
4,5-Dichloroveratrole	ND	ND	ND
3,4,5-Trichloroveratrole	ND	ND	ND
Tetrachloroveratrole	ND	ND	ND
Trichlorotrimethoxybenzene	0.68	0.39	0.68
5-Chlorovanillin	ND	ND	ND
6-Chlorovanillin	ND	ND	ND
5,6-Dichlorovanillin	ND	ND	ND
2-Chlorosyringaldehyde	ND	ND	ND
2,6-Dichlorosyringaldehyde	ND	ND	ND
4,5,6-Trichlorosyringol	ND	ND	ND

a. Detection Limit - 0.10 ppm

The mountain whitefish monitoring will fulfill the EEM requirement for a "game fish" type species. Population data would also still be gathered, but with an awareness of this species' mobility. Population data interpretation would have to be rigorous, with detailed examination of all data available from other studies such as NRBS. Cage studies would examine EROD induction, and could also be used for blood chemistry, since the stress of caging would be the same both upstream and downstream. Cooperative sex steroid work with other pulp mills in Alberta would involve capture of mountain whitefish from the ANC area, as well as from other areas in the Athabasca River and a reference river.

The forage fish study would provide fish population data that could be interpreted with more confidence. This recommendation stems from a conversation we recently had with Peter Hodson and Kelly Munkittrick of Environment Canada and Fisheries and Oceans Canada, respectively. They wondered why more pulp mills weren't considering forage species, given the difficulties with the more mobile large species. Since these two individuals are providing day-to-day input into EEM study design, their advice is worth noting.

Results of a preliminary investigation into forage fish compositon and relative abundance in the vicinity of the ANC mill are provided in Appendix 3. These investigations will be used to assist in the design of ANC's EEM program.

4.0 LITERATURE CITED

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APPENDIX 1

HISTOPATHOLOGY

III INDIVIDUAL REPORTS

EXPERIMENT: 93M (Spring) DATE: March 27, 1993

FISH ID: AC93WDSMTWT001 (93M001) TREATMENT: BIOMARKER SPRING 93

GROSS OBSERVATIONS: (field)

Green female. Whole spleen taken for histology. Sections of stomach, intestine, and a few pyloric caeca included in the sample. Excessive mesenteric fat observed in the fish. Anterior and posterior sections of the kidney taken for histology. (329 mm and 485 g) [F3?].

HISTOPATHOLOGY:

OVARY: Section containing immature ova with some eosinophilic yolk;
GILLS: Samples contained normal gills showing primary and secondary lamellae without damage;
HEART: NVL; (no visible lesions)
KIDNEY: Multiple nests of melanophages were seen;
MUSCLE: NVL;
PANCREAS: NVL;
STOMACH: NVL;
SMALL INTESTINE: No lesions seen; however, the epithelium had been severely traumatized;
LARGE INTESTINE: Slight increase in goblet cells (no significance);
GILLS: No lesions, both primary and secondary lamellae evaluated;

BONE: NVL;
CARTILAGE: NVL;
LYMPHOID TISSUE: Less than normal, but no necrosis, inflammation or neoplasia;
BUCCAL: Some vacuolar degeneration of some epithelium;
SPLEEN: White pulp not as prominent as usual;
LIVER: NVL;
HEMOPOIETIC SYSTEM: NVL;
THYROID: N/A;

No other lesions were seen.

MORPHOLOGICAL DIAGNOSIS:

1. Normal

COMMENTS:

A normal green female.

EXPERIMENT: 93M (Spring) DATE: March 27, 1993

FISH ID: AC93WDSMTWT002 (93M002) TREATMENT: BIOMARKER SPRING 93

GROSS OBSERVATIONS:

Resting female. Excessive mesenteric fat. Anterior and posterior sections of kidney taken for histology. Internal hemorrhage seen on the right ventral side between pectoral and pelvic fins. (417 mm and 1050 g) [F8?].

HISTOPATHOLOGY:

GONAD: Section containing no ova but a multilobulated tissue lined by a few cells resembling spermatogonia. Only one nest of spermatocytes was seen;
GILLS: Samples contained normal gills showing primary and secondary lamellae without damage;
HEART: NVL;
KIDNEY: Multiple nests of melanophages were seen;
MUSCLE: NVL;
PANCREAS: NVL;
STOMACH: NVL;
SMALL INTESTINE: No lesions seen;
LARGE INTESTINE: NVL;
BONE: NVL;

CARTILAGE: NVL;
LYMPHOID
TISSUE: Less than normal, but no necrosis, inflammation or neoplasia;
BUCCAL: NVL;
SPLEEN: Severe multifocal splenic hemosiderosis (of little significance);
LIVER: NVL;
HEMOPOIETIC
SYSTEM: NVL;
THYROID: N/A;

No other lesions were seen.

MORPHOLOGICAL DIAGNOSIS:

1. Severe multifocal splenic hemosiderosis

COMMENTS:

A normal resting male (please note this is not a female as was originally described). The splenic hemosiderosis means that iron metabolism (such as excessive breakdown of hemoglobin) had occurred in the past. This finding is not likely to have caused problems for the fish, but may have resulted from the old hemorrhage noted.

EXPERIMENT: 93M (Spring) DATE: March 27, 1993

FISH ID: AC93WDSMTWT003 TREATMENT: BIOMARKER SPRING 93
(93M003)

GROSS OBSERVATIONS:

Resting female. Excessive mesenteric fat. Anterior and posterior sections of the kidney taken for histology. (435 mm and 1095 g) [F8?].

HISTOPATHOLOGY:

GONAD: Section containing no ova but a multilobulated tissue lined by a few cells resembling spermatogonia. No nests of spermatocytes were seen;;
GILLS: Samples contained normal gills showing primary and secondary lamellae without damage;
HEART: NVL;
KIDNEY: Multiple nests of melanophages were seen;
MUSCLE: NVL;
PANCREAS: NVL;
STOMACH: NVL;
SMALL INTESTINE: No lesions seen; however, the epithelium had been severely traumatized;
LARGE INTESTINE: Slight increase in goblet cells (no significance);
GILLS: No lesions, both primary and secondary lamellae evaluated;
BONE: NVL;
CARTILAGE: NVL;

LYMPHOID
TISSUE: NVL;
BUCCAL: Some vacuolar degeneration of some epithelium;
SPLEEN: White pulp not very prominent; multiple lipomas noted;
LIVER: NVL;
HEMOPOIETIC
SYSTEM: NVL;
THYROID: N/A;

No other lesions were seen.

MORPHOLOGICAL DIAGNOSIS:

1. Multiple splenic lipomata

COMMENTS: A normal resting male. This is not a female as was described. The lipomata are benign tumors of little significance to the fish.

APPENDIX 2

CONTAMINANTS ANALYSES

1. DESCRIPTION OF SAMPLES

Samples received from SENTAR Consulting Ltd. Calgary, Alberta along with the analytical parameters requested are presented in Table 1.

TABLE 1: Samples Received, Matrices and Analysis Requested

LAB SAMPLE #	SAMPLE I.D.	MATRIX	ANALYSIS REQUESTED
E3-03-101-01A	AC93WDSMTWT001	FILLET	CDD/CDF, %L
E3-03-101-01B	AC93WDSMTWT001	LIVER	CDD/CDF, %L
E3-03-101-01C	AC93WDSMTWT001	BILE	CP, RA
E3-03-101-01D	AC93WDSMTWT001	MFO LIVER	EROD
E3-03-101-02A	AC93WDSMTWT002	FILLET	CDD/CDF, %L
E3-03-101-02B	AC93WDSMTWT002	LIVER	CDD/CDF, %L
E3-03-101-02C	AC93WDSMTWT002	BILE	CP, RA
E3-03-101-02D	AC93WDSMTWT002	MFO LIVER	EROD
E3-03-101-03A	AC93WDSMTWT003	FILLET	CDD/CDF, %L
E3-03-101-03B	AC93WDSMTWT003	LIVER	CDD/CDF, %L
E3-03-101-03C	AC93WDSMTWT003	BILE	CP, RA
E3-03-101-03D	AC93WDSMTWT003	MFO LIVER	EROD

Note: CDD/CDF = Dioxin and furan, %L = % Lipid, CP = Chlorophenols, RA = Resin and fatty acids, EROD = Ethoxyresorufin - o - deethylase

2. METHODS

2.1 Analysis of Chlorinated Phenols and Associated Derivatives

2.1.1 Analysis of Chlorinated Phenols and Associated Derivatives in Bile

The water was extracted and analyzed using MSOP 67.01.

2.2 Analysis of Diterpene Resin Acids and Fatty Acids

2.2.1 Analysis of Diterpene Resin Acids and Fatty Acids in Bile

The sediment was extracted and analyzed using MSOP 33.02.

2.3 Analysis for Polychlorinated Dibenzo-*para*-dioxins and Dibenzofurans

2.3.1 Polychlorinated Dibenzo-para-dioxins and Dibenzofurans in Fillet and Liver

The sediment was extracted, cleaned up, and analyzed using MSOP 20.00.

2.4 Ethyoxyresorufin - o - deethylase (EROD) in Liver

The livers were extracted and analyzed using MSOP 61.00.

3. REFERENCES

1. Environment Canada (1992). "Internal Quality Assurance Requirements for the Analysis of Dioxins in Environmental Samples". Report PES 1/RM/23. Available from: Environmental Protection Publications, Technology Development Branch, Conservation and Protection, Environment Canada, Ottawa, Ontario, K1A 0H3, 25 pp.

2. Environment Canada (1992). "Reference Method for the Determination of Polychlorinated Dibenzo-*para*-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) in Pulp and Paper Mill Effluents". Report EPS 1/RM/19. Available from: Environmental Protection Publications, Technology Development Branch, Conservation and Protection, Environment Canada, Ottawa, Ontario, K1A 0H3, 45 pp.

3. US-EPA Method 1613 Revision A (1990). "Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRMS/HRMS". Available form: US-EPA, Office of Water Regulations and Standards, 401 M Street SW, Washington, DC 20460, 42 pp.

4. RESULTS

Resin and Fatty Acids in Bile by GC/MSD
Please see attached.

Chlorinated Phenols in Bile by GC/MSD
Please see attached.

Dioxin and furan in Fillets and Liver
Please see attached.

EROD RESULTS:

LAB SAMPLE #	CLIENT I.D.	RESULTS pmol/min/mg
E3-03-101-01D	AC93WDSMTWT001	97
E3-03-101-02D	AC93WDSMTWT002	110
E3-03-03D	AC93WDSMTWT003	120
QC LOW		190
QC HIGH		2500

RESIN AND FATTY ACIDS ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR CONSULTANTS
MATRIX : BILE
LAB SAMPLE# : E3-03-101-01C-1
CLIENT I.D. : AC93WDSMTWT001 BILE
SAMPLE SIZE : 0.20 g

INSTRUMENT : HEWLETT PACKARD 5971A GC/MSD
ANALYSIS DATE : 31-MAR-93

DETECTION LIMIT: 0.010 ug/g (ppm)

	COMPOUND	CONCENTRATION ug/g (ppm)
FATTY ACIDS	ARACHIDIC ACID	ND
	LINOLEIC ACID	ND
	LINOLENIC ACID	ND
	MYRISTIC ACID	ND
	OLEIC ACID	ND
	PALMITIC ACID	ND
	STEARIC ACID	ND
	9,10-DICHLOROSTEARIC ACID	ND
	TOTAL FATTY ACIDS :	ND
RESIN ACIDS	ABIETIC ACID	ND
	DEHYDROABIETIC ACID	ND
	ISOPIMARIC ACID	ND
	LEVOPIMARIC ACID	ND
	NEOABIETIC ACID	ND
	PALUSTRIC ACID	ND
	PIMARIC ACID	ND
	SANDARACOPIMARIC ACID	ND
	12,14-DICHLORODEHYDROABIETIC ACID	ND
	12-CHLORODEHYDROABIETIC ACID [#2]	ND
	14-CHLORODEHYDROABIETIC ACID [#1]	ND
	TOTAL RESIN ACIDS :	ND
	TOTAL RESIN AND FATTY ACIDS :	ND

NOTES:

1.) ND = Not Detected, less than detection limit listed.
2.) The detection limit applies to all compounds listed.

QA/QC:

1.) To ensure resin acid extraction efficiency, the effluent was fortified with a surrogate compound prior to extraction. Based on in-house data, the average % recovery for:

O-methylpodocarpic acid 83% ± 18%

2.) To ensure resin acid derivatization efficiency, the final extracts were fortified with tricosanoic acid prior to methylation with diazomethane. Based on in-house recovery data, th average % recovery for:

Tricosanoic acid 102% ± 13%

RESIN AND FATTY ACIDS ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR CONSULTANTS
MATRIX : BILE
LAB SAMPLE# : E3-03-101-02C-1
CLIENT I.D. : AC93WDSMTWT002 BILE
SAMPLE SIZE : 0.20 g

INSTRUMENT : HEWLETT PACKARD 5971A GC/MSD
ANALYSIS DATE : 31-MAR-93

DETECTION LIMIT: 0.010 ug/g (ppm)

	COMPOUND	CONCENTRATION ug/g (ppm)
FATTY ACIDS	ARACHIDIC ACID	ND
	LINOLEIC ACID	ND
	LINOLENIC ACID	ND
	MYRISTIC ACID	ND
	OLEIC ACID	ND
	PALMITIC ACID	ND
	STEARIC ACID	ND
	9,10-DICHLOROSTEARIC ACID	ND
	TOTAL FATTY ACIDS :	ND
RESIN ACIDS	ABIETIC ACID	ND
	DEHYDROABIETIC ACID	ND
	ISOPIMARIC ACID	ND
	LEVOPIMARIC ACID	ND
	NEOABIETIC ACID	ND
	PALUSTRIC ACID	ND
	PIMARIC ACID	ND
	SANDARACOPIMARIC ACID	ND
	12,14-DICHLORODEHYDROABIETIC ACID	ND
	12-CHLORODEHYDROABIETIC ACID [#2]	ND
	14-CHLORODEHYDROABIETIC ACID [#1]	ND
	TOTAL RESIN ACIDS :	ND
	TOTAL RESIN AND FATTY ACIDS :	ND

NOTES:

1.) ND = Not Detected, less than detection limit listed.
2.) The detection limit applies to all compounds listed.

QA/QC:

1.) To ensure resin acid extraction efficiency, the effluent was fortified with a surrogate compound prior to extraction. Based on in-house data, the average % recovery for:

O-methylpodocarpic acid 83% ± 18%

2.) To ensure resin acid derivatization efficiency, the final extracts were fortified with tricosanoic acid prior to methylation with diazomethane. Based on in-house recovery data, th average % recovery for:

Tricosanoic acid 102% ± 13%

RESIN AND FATTY ACIDS ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR CONSULTANTS
MATRIX : BILE
LAB SAMPLE# : E3-03-101-03C-1
CLIENT I.D. : AC93WDSMTWT003 BILE
SAMPLE SIZE : 0.20 g

INSTRUMENT : HEWLETT PACKARD 5971A GC/MSD
ANALYSIS DATE : 31-MAR-93

DETECTION LIMIT: 0.010 ug/g (ppm)

	COMPOUND	CONCENTRATION ug/g (ppm)
FATTY ACIDS	ARACHIDIC ACID	ND
	LINOLEIC ACID	ND
	LINOLENIC ACID	ND
	MYRISTIC ACID	ND
	OLEIC ACID	ND
	PALMITIC ACID	ND
	STEARIC ACID	ND
	9,10-DICHLOROSTEARIC ACID	ND
	TOTAL FATTY ACIDS :	ND
RESIN ACIDS	ABIETIC ACID	ND
	DEHYDROABIETIC ACID	ND
	ISOPIMARIC ACID	ND
	LEVOPIMARIC ACID	ND
	NEOABIETIC ACID	ND
	PALUSTRIC ACID	ND
	PIMARIC ACID	ND
	SANDARACOPIMARIC ACID	ND
	12,14-DICHLORODEHYDROABIETIC ACID	ND
	12-CHLORODEHYDROABIETIC ACID [#2]	ND
	14-CHLORODEHYDROABIETIC ACID [#1]	ND
	TOTAL RESIN ACIDS :	ND
	TOTAL RESIN AND FATTY ACIDS :	ND

NOTES:

1.) ND = Not Detected, less than detection limit listed.
2.) The detection limit applies to all compounds listed.

QA/QC:

1.) To ensure resin acid extraction efficiency, the effluent was fortified with a surrogate compound prior to extraction. Based on in-house data, the average % recovery for:

O-methylpodocarpic acid 83% ± 18%

2.) To ensure resin acid derivatization efficiency, the final extracts were fortified with tricosanoic acid prior to methylation with diazomethane. Based on in-house recovery data, th average % recovery for:

Tricosanoic acid 102% ± 13%

CHLORINATED PHENOLS ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR CONSULTANTS
MATRIX : BILE
LAB SAMPLE# : E3-03-101-01
CLIENT I.D. : AC93WDSMTWT001
SAMPLE SIZE : 0.2 g

INSTRUMENT : HEWLETT PACKARD 5971A GC\MSD
ANAL. DATE : 01-APR-93
DETECTION LIMIT : 0.10 ug/g (ppm)

COMPOUND	Concentration ug/g (ppm)
2-Chlorophenol	ND
4-Chlorophenol	ND
2,4-Dichlorophenol	0.58
2,6-Dichlorophenol	ND
2,4,5-Trichlorophenol	ND
2,4,6-Trichlorophenol	1.7
2,3,4,6-Tetrachlorophenol	0.19
Pentachlorophenol	ND
4-Chlorocatechol	ND
3,4-Dichlorocatechol	ND
3,6-Dichlorocatechol	ND
3,5-Dichlorocatechol	ND
4,5-Dichlorocatechol	ND
3,4,5-Trichlorocatechol	ND
3,4,6-Trichlorocatechol	ND
Tetrachlorocatechol	ND
4-Chloroguaiacol	ND
3,4-Dichloroguaiacol	1.3
4,5-Dichloroguaiacol	0.49
4,6-Dichloroguaiacol	ND
3,4,5-Trichloroguaiacol	6.5
3,4,6-Trichloroguaiacol	0.19
4,5,6-Trichloroguaiacol	1.4
Tetrachloroguaiacol	4.1
4,5-Dichloroveratrole	ND
3,4,5-Trichloroveratrole	ND
Tetrachloroveratrole	ND
Trichlorotrimethoxybenzene	0.68
5-Chlorovanillin	ND
6-Chlorovanillin	ND
5,6-Dichlorovanillin	ND
2-Chlorosyringaldehyde	ND
2,6-Dichlorosyringaldehyde	ND
4,5,6-Trichlorosyringol	ND

SURROGATE	RECOVERY
2,4-Dichlorophenol-d3	68%
13C12-4-Chloroguaiacol	64%
13C12-5-Chlorovanillin	80%
13C12-4,5-Dichlorocatechol	86%
13C12-4,5,6-Trichloroguaiacol	82%
13C12-Pentachlorophenol	79%
13C12-Tetrachloroguaiacol	90%
13C12-Tetrachlorocatechol	85%
6-Bromo,2-naphthol	61%

NOTES:
1.) The recovery of the 6-bromo,2-naphthol surrogate is a measure of the method's hydrolysis efficiency.
2.) The recoveries of the d3 and 13C12 surrogates are a measure of the method's extraction efficiency.
3.) The sample results are NOT corrected for the surrogate recoveries.
4.) ND = Not Detected, less than detection limit listed.
NDR = Not detected due to ratios. The value reported is a conservative estimate of the total amount in the sample.

QA/QC : 5.) The detection limit applies to all compounds listed.

To ensure the chlorinated phenols extraction efficiency, the effluent was fortified with an 8-surrogate mix. Based on in-house data, the average recovery for:
13C12-4,5,6-Trichloroguaiacol 100% ± 24%

CHLORINATED PHENOLS ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR CONSULTANTS
MATRIX : BILE
LAB SAMPLE# : E3-03-101-02
CLIENT I.D. : AC93WDSMTWT002
SAMPLE SIZE : 0.2 g

INSTRUMENT : HEWLETT PACKARD 5971A GC\MSD
ANAL. DATE : 01-APR-93
DETECTION LIMIT : 0.10 ug/g (ppm)

COMPOUND	Concentration ug/g (ppm)
2-Chlorophenol	ND
4-Chlorophenol	ND
2,4-Dichlorophenol	0.39
2,6-Dichlorophenol	ND
2,4,5-Trichlorophenol	ND
2,4,6-Trichlorophenol	1.1
2,3,4,6-Tetrachlorophenol	ND
Pentachlorophenol	ND
4-Chlorocatechol	ND
3,4-Dichlorocatechol	ND
3,6-Dichlorocatechol	ND
3,5-Dichlorocatechol	ND
4,5-Dichlorocatechol	ND
3,4,5-Trichlorocatechol	ND
3,4,6-Trichlorocatechol	ND
Tetrachlorocatechol	ND
4-Chloroguaiacol	ND
3,4-Dichloroguaiacol	0.88
4,5-Dichloroguaiacol	0.29
4,6-Dichloroguaiacol	ND
3,4,5-Trichloroguaiacol	4.2
3,4,6-Trichloroguaiacol	ND
4,5,6-Trichloroguaiacol	0.88
Tetrachloroguaiacol	2.5
4,5-Dichloroveratrole	ND
3,4,5-Trichloroveratrole	ND
Tetrachloroveratrole	ND
Trichlorotrimethoxybenzene	0.39
5-Chlorovanillin	ND
6-Chlorovanillin	ND
5,6-Dichlorovanillin	ND
2-Chlorosyringaldehyde	ND
2,6-Dichlorosyringaldehyde	ND
4,5,6-Trichlorosyringol	ND

SURROGATE	RECOVERY
2,4-Dichlorophenol-d3	70%
13C12-4-Chloroguaiacol	68%
13C12-5-Chlorovanillin	80%
13C12-4,5-Dichlorocatechol	86%
13C12-4,5,6-Trichloroguaiacol	82%
13C12-Pentachlorophenol	80%
13C12-Tetrachloroguaiacol	88%
13C12-Tetrachlorocatechol	88%
6-Bromo,2-naphthol	63%

NOTES:
1.) The recovery of the 6-bromo,2-naphthol surrogate is a measure of the method's hydrolysis efficiency.
2.) The recoveries of the d3 and 13C12 surrogates are a measure of the method's extraction efficiency.
3.) The sample results are NOT corrected for the surrogate recoveries.
4.) ND = Not Detected, less than detection limit listed.
NDR = Not detected due to ratios. The value reported is a conservative estimate of the total amount in the sample.

QA/QC : 5.) The detection limit applies to all compounds listed.

To ensure the chlorinated phenols extraction efficiency, the effluent was fortified with an 8-surrogate mix. Based on in-house data, the average recovery for:
13C12-4,5,6-Trichloroguaiacol 100% ± 24%

CHLORINATED PHENOLS ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR CONSULTANTS
MATRIX : BILE
LAB SAMPLE# : E3-03-101-03
CLIENT I.D. : AC93WDSMTWT003
SAMPLE SIZE : 0.2 g

INSTRUMENT : HEWLETT PACKARD 5971A GC\MSD
ANAL. DATE : 01-APR-93
DETECTION LIMIT : 0.10 ug/g (ppm)

COMPOUND	Concentration ug/g (ppm)
2-Chlorophenol	ND
4-Chlorophenol	ND
2,4-Dichlorophenol	0.59
2,6-Dichlorophenol	ND
2,4,5-Trichlorophenol	ND
2,4,6-Trichlorophenol	1.3
2,3,4,6-Tetrachlorophenol	ND
Pentachlorophenol	ND
4-Chlorocatechol	ND
3,4-Dichlorocatechol	ND
3,6-Dichlorocatechol	ND
3,5-Dichlorocatechol	ND
4,5-Dichlorocatechol	ND
3,4,5-Trichlorocatechol	ND
3,4,6-Trichlorocatechol	ND
Tetrachlorocatechol	ND
4-Chloroguaiacol	ND
3,4-Dichloroguaiacol	1.5
4,5-Dichloroguaiacol	0.49
4,6-Dichloroguaiacol	ND
3,4,5-Trichloroguaiacol	6.6
3,4,6-Trichloroguaiacol	0.20
4,5,6-Trichloroguaiacol	1.2
Tetrachloroguaiacol	3.3
4,5-Dichloroveratrole	ND
3,4,5-Trichloroveratrole	ND
Tetrachloroveratrole	ND
Trichlorotrimethoxybenzene	0.68
5-Chlorovanillin	ND
6-Chlorovanillin	ND
5,6-Dichlorovanillin	ND
2-Chlorosyringaldehyde	ND
2,6-Dichlorosyringaldehyde	ND
4,5,6-Trichlorosyringol	ND

SURROGATE	RECOVERY
2,4-Dichlorophenol-d3	80%
13C12-4-Chloroguaiacol	80%
13C12-5-Chlorovanillin	94%
13C12-4,5-Dichlorocatechol	96%
13C12-4,5,6-Trichloroguaiacol	92%
13C12-Pentachlorophenol	91%
13C12-Tetrachloroguaiacol	100%
13C12-Tetrachlorocatechol	98%
6-Bromo,2-naphthol	72%

NOTES:
1.) The recovery of the 6-bromo,2-naphthol surrogate is a measure of the method's hydrolysis efficiency.
2.) The recoveries of the d3 and 13C12 surrogates are a measure of the method's extraction efficiency.
3.) The sample results are NOT corrected for the surrogate recoveries.
4.) ND = Not Detected, less than detection limit listed.
NDR = Not detected due to ratios. The value reported is a conservative estimate of the total amount in the sample.

QA/QC :
5.) The detection limit applies to all compounds listed.

To ensure the chlorinated phenols extraction efficiency, the effluent was fortified with an 8-surrogate mix. Based on in-house data, the average recovery for:
13C12-4,5,6-Trichloroguaiacol 100% ± 24%

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : BAKED SODIUM SULFATE
SAMPLE # : E3-03-101-MB
SAMPLE ID : METHOD BLANK
SAMPLE SIZE : 20.0 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (DB-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : N/A
MOISTURE : N/A

	Congener	Concent. (1) (pg/g)	DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND	0.1			
(3)	23-DiCDD	ND	0.1			
(3)	237-TriCDD	ND	0.1			
	2378-TCDD	ND	0.1	1	ND	0.047
*	12378-PeCDD	ND	0.1	0.5	ND	0.022
*	123478-HxCDD	ND	0.4	0.1	ND	0.019
*	123678-HxCDD	ND	0.4	0.1	ND	0.018
*	123789-HxCDD	ND	0.4	0.1	ND	0.019
	1234678-HpCDD	ND	0.6	0.01	ND	0.0029
	OCDD	ND	1.4	0.001	ND	0.000721
(3)	28-DiCDF	ND	0.6			
(3)	238-TriCDF	ND	0.1			
*	2378-TCDF	ND	0.1	0.1	ND	0.0065
*	12378-PeCDF	ND	0.1	0.05	ND	0.0013
*	23478-PeCDF	ND	0.1	0.5	ND	0.013
*	123478-HxCDF	ND	0.2	0.1	ND	0.0085
*	123678-HxCDF	ND	0.1	0.1	ND	0.0074
*	234678-HxCDF	ND	0.2	0.1	ND	0.0089
*	123789-HxCDF	ND	0.2	0.1	ND	0.012
	1234678-HpCDF	ND	0.4	0.01	ND	0.0018
	1234789-HpCDF	ND	0.6	0.01	ND	0.0032
	OCDF	ND	1.1	0.001	ND	0.000542
				Total TEQ	ND	0.19

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	0.1	0
TriCDD	ND	0.1	0
TCDD	ND	0.1	0
PeCDD	ND	0.1	0
HxCDD	ND	0.4	0
HpCDD	ND	0.6	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	0.6	0
TriCDF	ND	0.1	0
TCDF	ND	0.1	0
PeCDF	ND	0.1	0
HxCDF	ND	0.2	0
HpCDF	ND	0.5	0
Total PCDFs (Homologues)	ND		0

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	35%
13C12-2378-TCDD	1	64%
13C12-2378-TCDF	1	79%
13C12-12378-PeCDD	1	91%
13C12-12378-PeCDF	1	78%
13C12-23478-PeCDF	1	81%
13C12-123478-HxCDD	1	73%
13C12-123678-HxCDD	1	62%
13C12-123478-HxCDF	1	81%
13C12-123678-HxCDF	1	78%
13C12-234678-HxCDF	1	76%
13C12-123789-HxCDF	1	77%
13C12-1234678-HpCDD	1	74%
13C12-1234678-HpCDF	1	73%
13C12-1234789-HpCDF	1	69%
13C12-OCDD	2	54%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).

The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : FISH MUSCLE
SAMPLE # : E3-03-101-1A
SAMPLE ID : AC93WDSMTWT 001
SAMPLE SIZE : 20.2 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (DB-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : 5.9%
MOISTURE : 73.5%

	Congener	Concent. (1) (pg/g)	DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND	0.2			
(3)	23-DiCDD	ND	0.1			
(3)	237-TriCDD	ND	0.1			
	2378-TCDD	1.0	0.1	1	1.0	1.0
*	12378-PeCDD	ND	0.1	0.5	ND	0.036
*	123478-HxCDD	ND	0.2	0.1	ND	0.0095
*	123678-HxCDD	ND	0.2	0.1	ND	0.0100
*	123789-HxCDD	ND	0.2	0.1	ND	0.0097
	1234678-HpCDD	ND	0.3	0.01	ND	0.0017
	OCDD	ND	0.9	0.001	ND	0.000440
(3)	28-DiCDF	ND	0.2			
(3)	238-TriCDF	1.5	0.1			
*	2378-TCDF	3.6	0.1	0.1	0.36	0.36
*	12378-PeCDF	ND	0.1	0.05	ND	0.000227
*	23478-PeCDF	ND	0.1	0.5	ND	0.0023
*	123478-HxCDF	ND	0.2	0.1	ND	0.0082
*	123678-HxCDF	ND	0.2	0.1	ND	0.0077
*	234678-HxCDF	ND	0.2	0.1	ND	0.0096
*	123789-HxCDF	ND	0.3	0.1	ND	0.014
	1234678-HpCDF	ND	0.2	0.01	ND	0.0012
	1234789-HpCDF	ND	0.4	0.01	ND	0.0022
	OCDF	ND	0.7	0.001	ND	0.000363
				Total TEQ	1.4	1.5

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	0.1	0
TriCDD	ND	0.1	0
TCDD	ND	0.1	0
PeCDD	ND	0.1	0
HxCDD	ND	0.2	0
HpCDD	ND	0.3	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	0.2	0
TriCDF	0.6	0.1	1
TCDF	0.7	0.1	1
PeCDF	ND	0.1	0
HxCDF	ND	0.2	0
HpCDF	ND	0.3	0
Total PCDFs (Homologues)	1.2		2

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	92%
13C12-2378-TCDD	1	80%
13C12-2378-TCDF	1	81%
13C12-12378-PeCDD	1	97%
13C12-12378-PeCDF	1	91%
13C12-23478-PeCDF	1	87%
13C12-123478-HxCDD	1	80%
13C12-123678-HxCDD	1	81%
13C12-123478-HxCDF	1	101%
13C12-123678-HxCDF	1	92%
13C12-234678-HxCDF	1	85%
13C12-123789-HxCDF	1	86%
13C12-1234678-HpCDD	1	81%
13C12-1234678-HpCDF	1	93%
13C12-1234789-HpCDF	1	85%
13C12-OCDD	2	68%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).

The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : FISH LIVER
SAMPLE # : E3-03-101-1B
SAMPLE ID : AC93WDSMTWT 001
SAMPLE SIZE : 2.5 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (DB-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : 7.1%
MOISTURE : N/A

	Congener	Concent. (1) (pg/g)		DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND		2.8			
(3)	23-DiCDD	ND		2.4			
(3)	237-TriCDD	ND		2.3			
	2378-TCDD	NDR	(1.6)	1.2	1	1.6	1.6
*	12378-PeCDD	ND		1.3	0.5	ND	0.31
*	123478-HxCDD	ND		2.6	0.1	ND	0.13
*	123678-HxCDD	ND		2.7	0.1	ND	0.13
*	123789-HxCDD	ND		2.6	0.1	ND	0.13
	1234678-HpCDD	ND		6.6	0.01	ND	0.033
	OCDD	ND		44	0.001	ND	0.022
(3)	28-DiCDF	ND		3.1			
(3)	238-TriCDF		4.0	1.2			
*	2378-TCDF		3.1	0.7	0.1	0.31	0.31
*	12378-PeCDF	ND		1.6	0.05	ND	0.039
*	23478-PeCDF	ND		1.6	0.5	ND	0.39
*	123478-HxCDF	ND		1.9	0.1	ND	0.096
*	123678-HxCDF	ND		1.9	0.1	ND	0.094
*	234678-HxCDF	ND		2.1	0.1	ND	0.10
*	123789-HxCDF	ND		3.2	0.1	ND	0.16
	1234678-HpCDF	ND		3.6	0.01	ND	0.018
	1234789-HpCDF	ND		6.4	0.01	ND	0.032
	OCDF	ND		25	0.001	ND	0.013
					Total TEQ	1.9	3.6

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	2.4	0
TriCDD	ND	2.3	0
TCDD	ND	1.2	0
PeCDD	ND	1.3	0
HxCDD	ND	2.6	0
HpCDD	ND	6.6	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	3.1	0
TriCDF	ND	1.2	0
TCDF	ND	0.1	0
PeCDF	ND	1.6	0
HxCDF	ND	2.3	0
HpCDF	ND	5.0	0
Total PCDFs (Homologues)	ND		0

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	51%
13C12-2378-TCDD	1	76%
13C12-2378-TCDF	1	74%
13C12-12378-PeCDD	1	63%
13C12-12378-PeCDF	1	65%
13C12-23478-PeCDF	1	64%
13C12-123478-HxCDD	1	89%
13C12-123678-HxCDD	1	78%
13C12-123478-HxCDF	1	96%
13C12-123678-HxCDF	1	89%
13C12-234678-HxCDF	1	82%
13C12-123789-HxCDF	1	79%
13C12-1234678-HpCDD	1	77%
13C12-1234678-HpCDF	1	73%
13C12-1234789-HpCDF	1	72%
13C12-OCDD	2	45%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).

The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : FISH MUSCLE
SAMPLE # : E3-03-101-2A
SAMPLE ID : AC93WDSMTWT 002
SAMPLE SIZE : 20.0 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (DB-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : 5.4%
MOISTURE : 74.7%

	Congener	Concent. (1) (pg/g)	DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND	0.3			
(3)	23-DiCDD	ND	0.2			
(3)	237-TriCDD	ND	0.2			
	2378-TCDD	0.8	0.2	1	0.80	0.80
*	12378-PeCDD	ND	0.2	0.5	ND	0.047
*	123478-HxCDD	ND	0.5	0.1	ND	0.027
*	123678-HxCDD	ND	0.6	0.1	ND	0.030
*	123789-HxCDD	ND	0.6	0.1	ND	0.028
	1234678-HpCDD	ND	0.8	0.01	ND	0.0042
	OCDD	ND	2.8	0.001	ND	0.0014
(3)	28-DiCDF	ND	0.1			
(3)	238-TriCDF	1.9	0.1			
*	2378-TCDF	4.0	0.2	0.1	0.40	0.40
*	12378-PeCDF	ND	0.1	0.05	ND	0.0032
*	23478-PeCDF	ND	0.1	0.5	ND	0.032
*	123478-HxCDF	ND	0.4	0.1	ND	0.019
*	123678-HxCDF	ND	0.3	0.1	ND	0.017
*	234678-HxCDF	ND	0.4	0.1	ND	0.022
*	123789-HxCDF	ND	0.6	0.1	ND	0.029
	1234678-HpCDF	ND	0.4	0.01	ND	0.0022
	1234789-HpCDF	ND	0.9	0.01	ND	0.0047
	OCDF	ND	2.2	0.001	ND	0.0011
				Total TEQ	1.2	1.5

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	0.2	0
TriCDD	ND	0.2	0
TCDD	ND	0.2	0
PeCDD	ND	0.2	0
HxCDD	ND	0.6	0
HpCDD	ND	0.8	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	0.1	0
TriCDF	ND	0.1	0
TCDF	0.6	0.1	1
PeCDF	ND	0.1	0
HxCDF	ND	0.4	0
HpCDF	ND	0.7	0
Total PCDFs (Homologues)	0.6		1

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	67%
13C12-2378-TCDD	1	72%
13C12-2378-TCDF	1	77%
13C12-12378-PeCDD	1	53%
13C12-12378-PeCDF	1	62%
13C12-23478-PeCDF	1	58%
13C12-123478-HxCDD	1	92%
13C12-123678-HxCDD	1	93%
13C12-123478-HxCDF	1	104%
13C12-123678-HxCDF	1	104%
13C12-234678-HxCDF	1	90%
13C12-123789-HxCDF	1	93%
13C12-1234678-HpCDD	1	50%
13C12-1234678-HpCDF	1	77%
13C12-1234789-HpCDF	1	71%
13C12-OCDD	2	59%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).
The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : FISH MUSCLE
SAMPLE # : E3-03-101-2A (DUPLICATE)
SAMPLE ID : AC93WDSMTWT 002
SAMPLE SIZE : 20.0 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (DB-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : 5.4%
MOISTURE : 74.7%

	Congener	Concent. (1) (pg/g)	DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND	0.1			
(3)	23-DiCDD	ND	0.2			
(3)	237-TriCDD	ND	0.1			
	2378-TCDD	1.0	0.1	1	1.0	1.0
*	12378-PeCDD	ND	0.1	0.5	ND	0.026
*	123478-HxCDD	ND	0.3	0.1	ND	0.015
*	123678-HxCDD	ND	0.3	0.1	ND	0.015
*	123789-HxCDD	ND	0.3	0.1	ND	0.015
	1234678-HpCDD	ND	0.7	0.01	ND	0.0033
	OCDD	ND	5.8	0.001	ND	0.0029
(3)	28-DiCDF	ND	0.2			
(3)	238-TriCDF	1.8	0.1			
*	2378-TCDF	4.2	0.1	0.1	0.42	0.42
*	12378-PeCDF	ND	0.1	0.05	ND	0.0024
*	23478-PeCDF	ND	0.1	0.5	ND	0.024
*	123478-HxCDF	ND	0.3	0.1	ND	0.014
*	123678-HxCDF	ND	0.3	0.1	ND	0.014
*	234678-HxCDF	ND	0.3	0.1	ND	0.015
*	123789-HxCDF	ND	0.5	0.1	ND	0.023
	1234678-HpCDF	ND	0.3	0.01	ND	0.0017
	1234789-HpCDF	ND	0.6	0.01	ND	0.0030
	OCDF	ND	1.4	0.001	ND	0.000714
				Total TEQ	1.4	1.6

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	0.2	0
TriCDD	ND	0.1	0
TCDD	ND	0.1	0
PeCDD	ND	0.1	0
HxCDD	ND	0.3	0
HpCDD	ND	0.7	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	0.2	0
TriCDF	ND	0.1	0
TCDF	0.7	0.1	1
PeCDF	ND	0.1	0
HxCDF	ND	0.3	0
HpCDF	ND	0.5	0
Total PCDFs (Homologues)	0.7		1

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	58%
13C12-2378-TCDD	1	70%
13C12-2378-TCDF	1	72%
13C12-12378-PeCDD	1	55%
13C12-12378-PeCDF	1	60%
13C12-23478-PeCDF	1	54%
13C12-123478-HxCDD	1	82%
13C12-123678-HxCDD	1	85%
13C12-123478-HxCDF	1	100%
13C12-123678-HxCDF	1	87%
13C12-234678-HxCDF	1	94%
13C12-123789-HxCDF	1	83%
13C12-1234678-HpCDD	1	90%
13C12-1234678-HpCDF	1	99%
13C12-1234789-HpCDF	1	49%
13C12-OCDD	2	72%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).

The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : FISH MUSCLE
SAMPLE # : E3-03-101-3A
SAMPLE ID : AC93WDSMTWT 003
SAMPLE SIZE : 20.0 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (D8-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : 5.7%
MOISTURE : 74.1%

	Congener	Concent. (1) (pg/g)	DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND	0.4			
(3)	23-DiCDD	ND	0.4			
(3)	237-TriCDD	ND	0.2			
	2378-TCDD	6.3	0.2	1	6.3	6.3
*	12378-PeCDD	ND	0.1	0.5	ND	0.035
*	123478-HxCDD	ND	0.3	0.1	ND	0.016
*	123678-HxCDD	ND	0.3	0.1	ND	0.016
*	123789-HxCDD	ND	0.3	0.1	ND	0.016
	1234678-HpCDD	ND	0.5	0.01	ND	0.0024
	OCDD	ND	2.7	0.001	ND	0.0013
(3)	28-DiCDF	ND	0.3			
(3)	238-TriCDF	1.9	0.2			
*	2378-TCDF	19	0.2	0.1	1.9	1.9
*	12378-PeCDF	ND	0.2	0.05	ND	0.0048
*	23478-PeCDF	ND	0.2	0.5	ND	0.048
*	123478-HxCDF	ND	0.4	0.1	ND	0.019
*	123678-HxCDF	ND	0.3	0.1	ND	0.016
*	234678-HxCDF	ND	0.4	0.1	ND	0.019
*	123789-HxCDF	ND	0.5	0.1	ND	0.027
	1234678-HpCDF	ND	0.4	0.01	ND	0.0022
	1234789-HpCDF	ND	0.8	0.01	ND	0.0042
	OCDF	ND	1.3	0.001	ND	0.000656
				Total TEQ	8.2	8.4

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	0.4	0
TriCDD	ND	0.2	0
TCDD	ND	0.2	0
PeCDD	ND	0.1	0
HxCDD	ND	0.3	0
HpCDD	ND	0.5	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	0.3	0
TriCDF	0.6	0.2	1
TCDF	0.6	0.1	1
PeCDF	ND	0.2	0
HxCDF	ND	0.4	0
HpCDF	ND	0.6	0
Total PCDFs (Homologues)	1.2		2

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	49%
13C12-2378-TCDD	1	69%
13C12-2378-TCDF	1	67%
13C12-12378-PeCDD	1	67%
13C12-12378-PeCDF	1	64%
13C12-23478-PeCDF	1	63%
13C12-123478-HxCDD	1	81%
13C12-123678-HxCDD	1	81%
13C12-123478-HxCDF	1	77%
13C12-123678-HxCDF	1	81%
13C12-234678-HxCDF	1	72%
13C12-123789-HxCDF	1	74%
13C12-1234678-HpCDD	1	73%
13C12-1234678-HpCDF	1	74%
13C12-1234789-HpCDF	1	68%
13C12-OCDD	2	56%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).

The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

DIOXIN ANALYSIS REPORT
ENVIROTEST LABORATORIES
PULP AND PAPER DIVISION

PROJECT : SENTAR
MATRIX : FISH LIVER
SAMPLE # : E3-03-101-38
SAMPLE ID : AC93WDSMTWT 003
SAMPLE SIZE : 10.0 g (WET WEIGHT)

GC COLUMN : HP-Ultra 2 (DB-5), 50m x 0.2mm
INSTRUMENT : KRATOS CONCEPT IS32 HRMS
ANAL. DATE : 11-Apr-93
LIPIDS : 7.0%
MOISTURE : N/A

	Congener	Concent. (1) (pg/g)	DL (pg/g)	I-TEF (NATO)	TEQ (ND=0) (pg/g)	TEQ (ND=½DL) (pg/g)
(3)	27/28-DiCDD	ND	0.1			
(3)	23-DiCDD	ND	0.2			
(3)	237-TriCDD	ND	0.1			
	2378-TCDD	0.7	0.7	1	0.70	0.70
*	12378-PeCDD	ND	150	0.5	ND	37
*	123478-HxCDD	ND	1.5	0.1	ND	0.075
*	123678-HxCDD	ND	1.4	0.1	ND	0.071
*	123789-HxCDD	ND	1.5	0.1	ND	0.073
	1234678-HpCDD	ND	1.4	0.01	ND	0.0068
	OCDD	ND	8.3	0.001	ND	0.0041
(3)	28-DiCDF	ND	0.4			
(3)	238-TriCDF	3.0	0.2			
*	2378-TCDF	3.0	1.1	0.1	0.30	0.30
*	12378-PeCDF	ND	47	0.05	ND	1.2
*	23478-PeCDF	ND	100	0.5	ND	12
*	123478-HxCDF	ND	1.3	0.1	ND	0.066
*	123678-HxCDF	ND	1.2	0.1	ND	0.060
*	234678-HxCDF	ND	1.4	0.1	ND	0.071
*	123789-HxCDF	ND	2.1	0.1	ND	0.10
	1234678-HpCDF	ND	1.5	0.01	ND	0.0075
	1234789-HpCDF	ND	2.6	0.01	ND	0.013
	OCDF	ND	13	0.001	ND	0.0065
				Total TEQ	1.0	51

Homologue (2)	Concent. (1) (pg/g)	DL (pg/g)	NP
DiCDD	ND	0.2	0
TriCDD	ND	0.1	0
TCDD	ND	0.7	0
PeCDD	ND	150	0
HxCDD	ND	1.5	0
HpCDD	ND	1.4	0
Total PCDDs (Homologues)	ND		0
DiCDF	ND	0.4	0
TriCDF	ND	0.2	0
TCDF	ND	0.1	0
PeCDF	ND	75	0
HxCDF	ND	1.5	0
HpCDF	ND	2.0	0
Total PCDFs (Homologues)	ND		0

SURROGATE	Amount added (ng)	RECOVERY
13C12-27-DiCDD	1	99%
13C12-2378-TCDD	1	90%
13C12-2378-TCDF	1	98%
13C12-12378-PeCDD	1	88%
13C12-12378-PeCDF	1	52%
13C12-23478-PeCDF	1	82%
13C12-123478-HxCDD	1	94%
13C12-123678-HxCDD	1	97%
13C12-123478-HxCDF	1	105%
13C12-123678-HxCDF	1	92%
13C12-234678-HxCDF	1	87%
13C12-123789-HxCDF	1	83%
13C12-1234678-HpCDD	1	55%
13C12-1234678-HpCDF	1	55%
13C12-1234789-HpCDF	1	56%
13C12-OCDD	2	42%

Notes:
1.- Results are corrected for surrogate recovery.
2.- Total Homologue results do NOT include the 2,3,7,8- isomers.
3.- Concentrations reported for Di/TriCDD's are only an indication of their presence, and may not be accurate.

* Value represents maximum possible amount. This isomer might co-elute with other isomer(s).

The following abbreviations are used:
DL = Detection Limit.
ND = Not Detected.
TEQ = 2,3,7,8-TCDD Toxic Equivalency
NDR = Not Detected due to incorrect isotope Ratio.
N/A = Not Applicable.
NP = Number of Analyte Peaks.
DPE = DiPhenyl Ether interference.
I-TEF = International Toxic Equivalency Factor.

APPENDIX 3

FORAGE FISH COLLECTIONS

APPENDIX 3

FORAGE FISH COLLECTIONS

Forage fish collections were conducted on May 13 and 14, 1993 on a reach of the Athabasca River extending from about 3 km upstream of the ANC mill to 5 km below the mill. Average daily flow in the Athabasca River at at Windfall at the time of sampling ranged from 271 to 441 m^3/s (Alberta Environmental Protection, River Forecast Centre, pers. comm.) Ten sites were selected and sampled using a 7 m long by 1.2 m deep seine net with a mesh size of 6 mm (stretch measure) (Figure A1). Backpack electrofishing was done at one site (Site 1). The habitat type at most sampling sites was low current velocity, backwater areas with either a rock (cobble, pebble, gravel) or sand/silt substrate (Table A1). Run habitat was sample at two sites (Sites 8 and 10) where cobble and pebble were the dominant substrate. Average water depth at sampling sites ranged from 25 - 45 cm; the maximum depth sampled was 75 cm. Incidental water temperature measurements, using a pocket thermometer, ranged from 17 to 19 C during the sampling program.

Ten species of fish were captured within the study area (Table A2). Of these, seven species, pearl dace (*Semotilus margarita*), longnose dace (*Rhinichthys cataractae*), spottail shiner (*Notropis hudsonius*), trout-perch (*Percopsis omiscomaycus*), lake chub (*Couesius plumbeus*), emerald shiner (*Notropis atherinoides*) and brook stickleback (*Culaea inconstans*) are considered forage fish. Two species of sportfish, mountain whitefish (*Prosopium williamsoni*) and northern pike (*Esox lucius*) were also captured while only one coarse fish species, longnose sucker (*Catostomus catostomus*) was recorded.

Longnose sucker were the most abundant species taken followed by pearl dace and longnose dace. Longnose sucker were recorded at all sites with the exception of Site 5. This species was most abundant at Sites 6 and 7, both backwater areas. All longnose suckers captured were juvenile fish, probably yearling and two-year olds, based on size. Pearl dace were the next most common species captured and were the most abundant at Sites 6 and 8. Longnose dace were the third most abundant fish taken with the highest catch-per-unit effort for this species recorded at Sites 8 and 10. All other species were found in relatively low numbers with only one or two individuals recorded in most cases. All mountain whitefish captured were yearlings (75 - 90 mm fork length) with parr marks evident on all fish. The solitary northern pike captured was a juvenile approximately 225 mm in length.

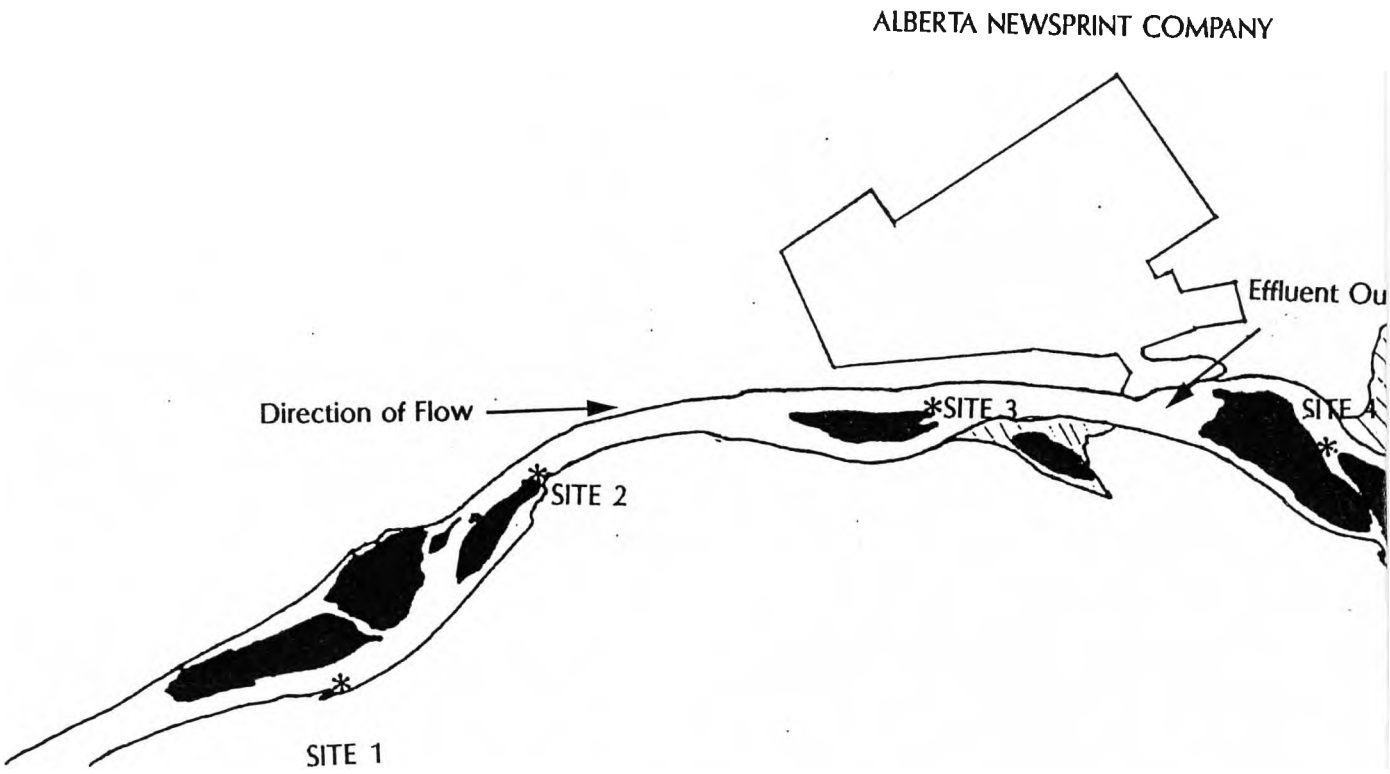

Figure A1. Fish collection sampling sites, May 13 and 14, 1993.

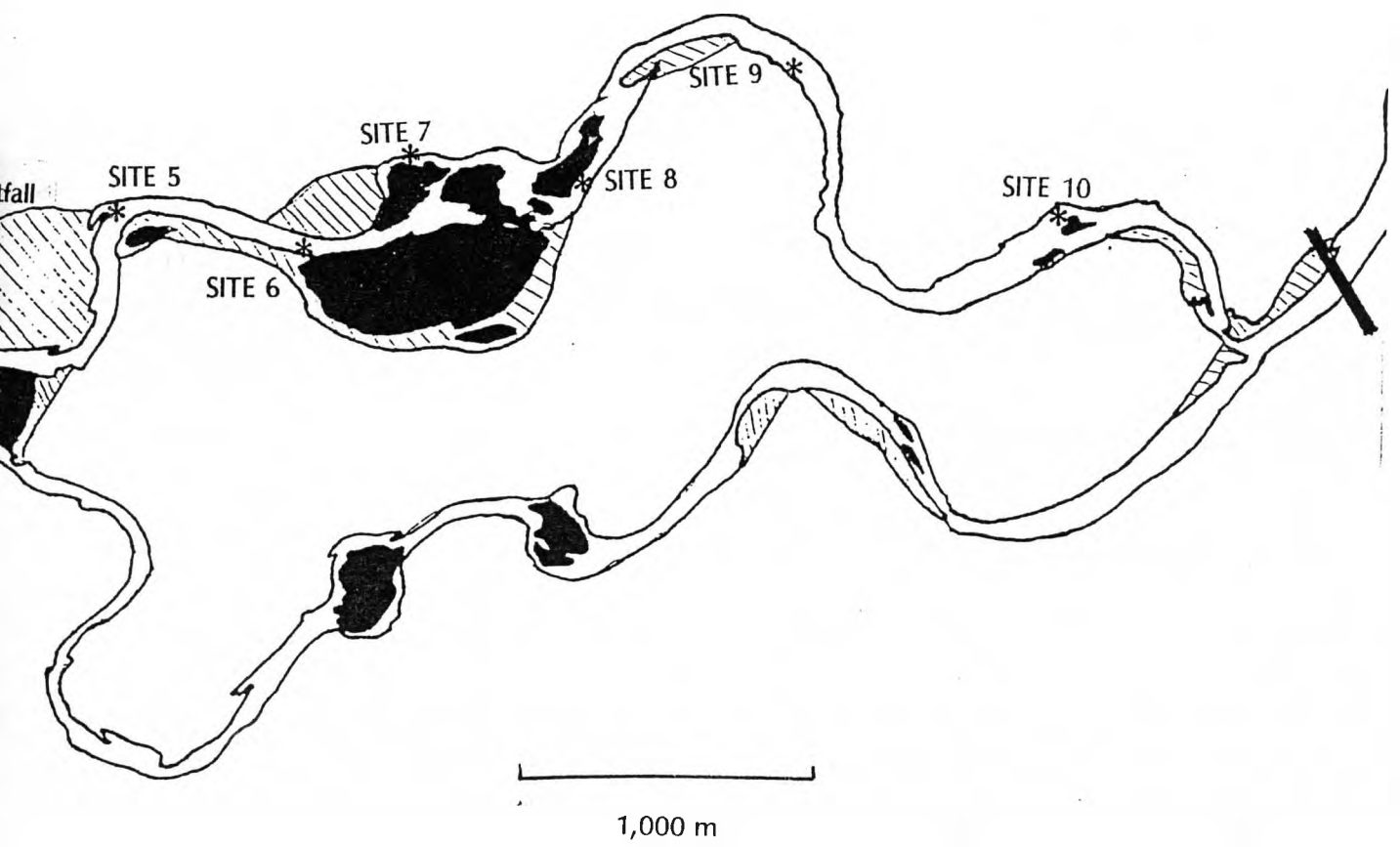
tfall
SITE 5
SITE 6
SITE 7
SITE 8
SITE 9
SITE 10
1,000 m

Table A1. Habitat characteristics of seine sites, Athabasca River, May 13 and 14 1993.

Site	Habitat Type	Substrate Composition	Average Depth (cm)
1	Backwater	30% cobble, 65% pebble, 5% gravel	25 - 45
2	Backwater	40% cobble, 60% pebble	30
3	Backwater	80% cobble, 20% pebble	40
4	Backwater	100% sand/silt	35
5	Backwater	100% sand/silt	45
6	Backwater	100% sand/silt	45
7	Backwater	30% cobble, 70% pebble,	40
8	Run	45% cobble, 55% pebble	30
9	Backwater	100% sand/silt	30 - 45
10	Run	5% cobble, 95% pebble	30

Table A2. Number of fish and catch-per-unit effort (CPUE) (number/100 m^2) from the Athabasca River near Whitecourt, Alberta, May 13 and 14, 1993.

Site	Area Fished (m^2)	Number of Fish (CPUE)									
		MNWH	PRDC	LNSC	LNDC	SPSH	TRPR	LKCH	NRPK	EMSH	BRST
1	461	2(0.4)	19(4.1)	18(3.9)	1(0.2)	1(0.2)	0	0	0	0	0
2	108	0	0	3(2.8)	0	0	0	0	0	0	0
3	585	0	0	13(2.2)	6(1.0)	0	0	0	0	0	0
4	258	0	0	2(0.8)	2(0.8)	0	2(0.8)	1(0.4)	0	0	0
5	70	0	0	0	0	0	0	0	0	0	0
6	132	9(6.8)	11(8.3)	157(118.9)	1(0.8)	3(2.3)	0	0	1(0.4)	0	0
7	216	0	0	45(20.8)	0	0	0	0	0	0	0
8	84	0	11(13.1)	4(4.8)	19(22.6)	0	0	0	0	0	0
9	453	0	20(4.4)	4(0.9)	5(1.1)	12(2.7)	0	0	0	1(0.2)	1(0.2)
10	132	1(0.8)	6(4.9)	12(9.1)	3(2.3)	0	0	0	0	0	0
Total	**2499**	**12(0.5)**	**67(2.7)**	**258(10.3)**	**37(1.5)**	**16(0.6)**	**2(0.1)**	**1(<0.1)**	**1(<0.1)**	**1(<0.1)**	**1(<0.1)**

MNWH Mountain Whitefish
PRDC Pearl Dace
LNSC Longnose Sucker
LNDC Longnose Dace
SPSH Spottail Shiner
TRPR Trout-Perch
LKCH Lake Chub
NRPK Northern Pike
EMSH Emerald Shiner
BRST Brook Stickleback

In terms of using forage fish as a target species for EEM, two candidate species are available, pearl dace and longnose dace. These species were generally abundant enough to provide a large enough sample size to meet EEM requirements. Two approaches to EEM are suggested by the forage fish collection data. The first and preferred approach would be to collect forage fish "in situ" at sites both upstream and downstream of ANC. Depending on the seasonal abundance of the forage fish target species, a sufficient number of fish could be collected at an exposure site(s) just below the ANC outfall. An upstream site (between Windfall and ANC) would be established as a control. If suitable numbers are not found at a site in close proximity to the effluent outfall, then an alternative would be to proceed with caged experiments. Field crews would be duly prepared to proceed with cage experiments should this prove to be the better alternative.