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Chemical Characterization and Toxicological Assessment of Kraft Pulp Mill Fiber Waste as a Feedstuff for Beef Cattle

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Pulp fiber waste (PFW), a byproduct of pulp and paper mills has been proposed as an energy source for ruminants (Croy and Rode, 1988). Chemical analysis of pulp and paper effluents have identified numerous compounds with toxicological potentials (McKean, 1980; Kringstad and Lindstrom, 1984). Certain fractions of pulp effluents show mutagenic activity *in vitro* in both bacterial (McKague *et al.*, 1981; Nestmann *et al*, 1979) and yeast (Nestmann and Lee, 1983) assays. The presence of resin acids, chlorinated resin acids, chlorinated phenolics and guaiacols derived from the kraft process and sequential bleaching stages contribute to the effluent's toxicity and mutagenicity (Walden and Howard, 1981; Kubic and Jackson, 1981; McKague, 1981). In addition, various dioxin and furan isomers have been identified in wood products (Van Strum and Merrell, 1987). Similar biologically active chemical toxins also may be present in PFW. This possibility presents a potential hazard if PFW is to be used as an alternative feed source for meat or milk producing animals. No studies to date have characterized the chemical composition of PFW in terms of its toxic potential. The present study assesses two sources of PFW in a short-term mutagenicity test and a long-term animal feeding trial to determine the toxicological potential of PFW.

MATERIALS AND METHODS

PFW samples were obtained from Weyerhaeuser Canada Ltd., Kamloops, B.C. (K PFW) and Canfor Ltd., Prince George, B.C. (PG PFW). Subsamples were subjected to a standard nitric perchloric acid and hydrogen chloride digest, and analyzed for heavy metals and trace elements with ICAP AES (Inductively Coupled Argon Plasma Atomic Emission Spectroscopy). Cotton cellulose was the standard reference material. Total resin acids and chlorinated phenolics were extracted from wet PFW samples. Subsamples were adjusted to pH 8.0 (neutral fraction) and extracted twice with diethyl ether:methanol (8:1). The remaining pulp fiber was acidified with sulfuric acid to pH 1.0 (acidic fraction). Both the neutral and acidic fractions were extracted into dichloromethane which was subsequently replaced with iso-octane by rotary evaporation. The iso-octane extracts were methylated, adsorbed onto a Florisil column and eluted with petroleum ether (fraction 1) and petroleum ether:ethyl acetate

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(98:2) (fraction 2). Both fractions were injected into a gas liquid chromatograph (GLC) (HP 5880A) equipped with a capillary column (DB1) and flame ionization detector. Resin acids were detectable above 200 ppb. Both fractions were chromatographed on a second capillary column gas liquid chromatograph (HP 5710A) equipped with an electron capture detector. Chlorinated guaiacols were detectable above 10 ppb. A PFW sample spiked with a mixture of resin acids, chlorinated guaiacols and terpene/phenols assured quality control. A reagent blank checked for interferences.

Mann Testing Laboratories (Mississauga, Ontario, Canada) analyzed the PFW and tissue samples for dioxins and furans. Homogenized samples were digested in concentrated HCl and extracted with 1% dichloromethane in hexane. All samples were subjected to a multi-adsorbent column cleanup (Parski and Nestrick, 1980) to reduce sample background. The samples were analyzed by GLC under electron impact conditions at a 300-500 molecular equivalent range. Compounds were identified on the basis of their specific retention times, full scan analysis and parent ion cluster ratios of the sample and standard solutions. Quality control was assured by spiking samples with carbon 13 labelled tetrachlorodibenzodioxin (C13-TCDD) and octachlorodibenzodioxin (C13-OCDD).

Mutagenicity assays were performed on the water soluble and organic soluble fractions of PFW from the two pulp mills. Water soluble fractions (10 g in 200 ml deionized water) and organic fractions (10 g in 200 ml chloroform:methanol (4:1)) were obtained by mixing the slurry solutions in a shaker-incubator for 24 hr at 25°C. Slurries were gravity filtered. Water soluble fractions were freeze-dried and organic fractions were concentrated in dimethylsulfoxide (DMSO) by rotary evaporation. Aqueous samples were sterilized by passing the solution through a membrane filter (0.2 µm, Nalgene) prior to assay. The Ames test was performed, without metabolic activation, with histidine-dependent mutant strains (TA 98, TA 100 and TA 1537) of *Salmonella typhimurium* obtained from B.C. Research, Vancouver, B.C. and handled according to Nestmann *et al.*, 1980. All colonies were counted manually or when appropriate by a colony counter. Controls included the number of spontaneous revertants in sterile water, DMSO, sodium azide, 9-aminocridine and 2-nitrofluorene. Samples are considered mutagenic if there is a 2-3 fold increase in revertant colonies over that in the negative controls.

In the animal feeding study, 27 Hereford-cross long yearling steers (initial body weight: 398 ± 65 kg) were fed either a control ration or a ration containing 44.4% PFW on a dry matter basis from either of two different Kraft pulp mills for 63 days. A completely randomized design consisting of 3 animals per pen, replicated 3 times was used. The PFW was ensiled with barley, whey and urea. This PFW silage was mixed with alfalfa/grass hay, barley, canola and supplemented with vitamins and minerals. Control animals received a ration consisting of 80% barley and 20% hay. *Ad libitum* pen intakes and individual body weights were measured weekly. Following the feeding trial, a veterinarian examined the animals. Liver biopsy samples (0.5 g) taken randomly from each of the control, K PFW and PG PFW treatment groups, were analyzed for dioxins, furans, resin

acids and chlorinated phenols.

RESULTS AND DISCUSSION

Heavy metals and other trace element concentrations in both PFW samples were below the tolerance level for beef cattle (NAS-NRC, 1984) (Table 1). However, since the PFW was diluted in the total ration, the mineral contamination due to the PFW was minimized.

Relative moisture content, total resin acids and chlorinated guaiacol composition for the PFW samples are reported in Table 2. To some extent the lower moisture content of the K PFW sample contributed to higher concentrations of the resin acids compared to the PG PFW samples. However despite the relative difference in moisture between the samples, levopimaric, abietic and neoabietic acid concentrations were particularly elevated in the K PFW. According to McKean (1980), the 96hr LC_{50} values of coho salmon (a standard toxicity bioassay where 50% of the test population survives after a 96 hour exposure to a known concentration of a certain substance) indicate approximately 80% of the toxicity associated with the total mill effluent is attributed to the resin acid content of which pimaric acids are generally more toxic than abietic acids (Leach and Thakore, 1974). The resin acids identified in the PFW samples corresponded to those found in pulp and paper effluents (Oikai and Holmbom, 1986) and were present in concentrations above levels known to be hazardous to fish and other aquatic organisms (Hemingway and Greaves, 1973). Relativity less is known about the toxicity of resin acids in mammalian species (Kubik and Jackson, 1981).

In addition to the resin acids detected in the PFW, K PFW contained tetrachloroguaiacol in substantially greater amounts than the PG PFW (Table 2). Tetrachloroguaicol and trichloroguaiacol, derived from the chlorination of lignin, are toxic to fish (McKean, 1980). Unlike resin acids, chlorinated guaiacols persist in the environment and may eventually lead to bioaccumulation in the ecosystem.

The K PFW showed 9.3 ppb of total tetrachlorodibenzofurans (TCDF). No dioxins or furans were identified in the PG PFW sample with a detection limit of 200-500 ppt (Table 3). The expressed values refer to the cumulative total of a number of different tetrachlorinated furan isomers. Since each isomer varies in its relative toxicity (McConnell and Moore, 1979), assessing the overall toxicity due to the TCDFs in the K PFW is difficult. Relatively high detection limits for tetrachlorodibenzodioxins (2,3,7,8-TCDD) (K PFW = 5.0 ppb and PG PFW =0.2 ppb) in this study preclude any estimation of their presence in the PFW samples. Although the World Health Organization has suggested that man is less sensitive to polychlorinated dibenzo-dioxins and polychlorinated dibenzo-furans than other mammalian species (Nygren *et al.*, 1986), both American and Canadian agencies have classified TCDD as a possible carcinogen.

Various concentrations of aqueous and organic fractions from the PFW samples were tested for mutagenicity with three strains of *Salmonella typhimurium* using the Ames test. When tested without metabolic activation, no mutagenicity was observed in either

Table 1 Mineral profiles of pulp mill fiber waste [1]

ELEMENT[3]	Source of PFW[2] K PFW	PG PFW
ALUMINUM (1000)	600	657
ARSENIC (50)	0.8	0.4
CADMIUM (0.5)	0.3	0.4
COBALT (5)	1	< 0.6
COPPER (115)	2	5
MERCURY (2)	0.1	0.03
LEAD (30)	< 5	< 3
SELENIUM (2)	< 0.1	< 0.06

1. values (ppm) represent the mean of samples analyzed in duplicate and expressed on a dry matter basis.
2. PG PFW = Prince George pulp fiber waste; K PFW = Kamloops pulp fiber waste
3. figures in brackets represent tolerance levels (ppm) for beef cattle (NAS-NRC, 1984)

Table 2 Concentration of resin acids and chlorinated compounds in pulp fiber waste [1]

Compound	PG PFW	K PFW
RESIN ACIDS[2]		
PIMARIC	40.92	75.16
SANDARACOPIMARIC	6.68	13.00
ISOPIMARIC	62.10	97.30
LEVOPIMARIC	17.02	81.11
DEHYDROABIETIC	46.95	68.50
ABIETIC	50.62	246.35
NEOABIETIC	2.84	13.65
CHLORINATED GUAIACOLS[3]		
TRI-Cl GUAIACOL	0.025	0.023
TETRA-Cl GUAIACOL	0.096	0.311
% MOISTURE	76.67	62.84
pH	8.0	9.0

1. values (ppm), on a wet weight basis, represent single samples. PG PFW = Prince George pulp fiber waste; K PFW = Kamloops pulp fiber waste
2. detection limit for resin acids in PFW is 0.200 ppm
3. detection limit for chlorinated guaiacols in PFW is 0.010 ppm

fraction from both PFW sources (Table 4). In previous studies, metabolic activation did not enhance mutagenicity in pulp mill effluents (McKague *et al.*, 1981). Pulp mill effluents have shown mutagenic activity in the *Salmonella*/mammalian-microsome test (McKague *et al.*, 1981; Nestmann *et al.* 1979; 1980). While pure

standards of resin acids including abietic, levopimaric and pimaric acids showed no mutagenic activity with the Ames test, neoabietic acid did show a dose-related mutagenic response (Nestmann *et al.*, 1979). In this study K PFW showed no mutagenicity even though neoabietic acid was detectable. Halogenated carcinogens such as TCDD failed to induce mutagenicity in short-term mutagenicity assays (Ames and McCann, 1981). The lack of mutagenicity in our study may partially explained by relatively low levels of mutagenic compounds compared to other toxic, nonmutagenic substances present in the PFW.

The average feed intake (FI) (kg/day), average daily gain (ADG) (kg/day) and feed conversion ratios (FCR) (feed/gain) during the 63 day trial were respectively: control 11.1, 1.9, 5.5; PG PFW 10.4, 1.6, 6.6; K PFW 9.8, 1.5, 6.7. Weight gains during the measured period were linear and are described by the following equations: control $y = 396.97 + 12.57x$ ($r^2 = 0.997$), PG PFW $y = 397.67 + 11.01x$ ($r^2 = 0.991$), K PFW $y = 408.15 + 10.01x$ ($r^2 = 0.996$). Although ADG

Table 3 Dioxin and furan detection levels and concentrations in PFW[1]

COMPOUND[2] (Cumulative isomer)	K PFW DR	K PFW MDL	PG PFW DR	PG PFW MDL
TETRA-CDD (0.1)	-	5.0	-	0.2
PENTA-CDD (0.1)	-	1.8	-	0.2
HEXA-CDD (0.1)	-	1.7	-	0.3
HEPTA-CDD (0.3)	-	1.0	-	0.5
OCTA-CDD (0.3)	-	1.0	-	0.5
TETRA-CDF (0.1)	9.3	1.2	-	0.2
PENTA-CDF (0.1)	-	1.8	-	0.2
HEXA-CDF (0.1)	-	1.7	-	0.3
HEPTA-CDF (0.3)	-	1.0	-	0.5
OCTA-CDF (0.3)	-	1.0	-	0.5

1. values, on a wet weight basis, represent a single measurement. DR = detectable residue (ppb); MDL = method detection limit (ppb); - = none detected; CDD = chlorodibenzodioxin; CDF = chlorodibenzofuran.
2. values in brackets indicate background detection limit for an external blank where recovery was 78% for C13-TCDD and 96% for C13-OCDD. Sample matrix recoveries were 61% and 47% for C13-TCDD and C13-OCDD in K PFW; sample matrix recoveries were 95% and 85% for C13-TCDD and C13-OCDD in the PG PFW.

was significantly higher ($P < 0.05$) in the control animals compared with the animals fed PFW, all groups showed respectable weight gains. No significant difference ($P < 0.05$) in FI or FCR between groups was measured. No visual signs of toxicity in the treatment animals were observed during the trial. Other studies also report no change in weight gain, feed consumption and milk production following the feeding of artifically high levels of TCDD in the form

Table 4 Number of revertant colonies[1] of *Salmonella typhimurium* induced by pulp fiber waste extracts

SAMPLE	BACTERIAL STRAIN TA 98	TA 100	TA 1537
CONTROLS:			
DMSO (100 µl)	45	139	10
(500 µl)	32	49	6
WATER (100 µl)	-	172	6
(200 µl)	-	-	-
(500 µl)	35	235	-
(700 µl)	-	-	-
(1000 µl)	-	171	9
(2000 µl)	41	196	-
2-NITROFLUORENE (50 µg)	1363	-	-
SODIUM AZIDE (10 µg)	-	2205	-
9-AMINOACRIDE (100 µg)	-	-	1420

KAMLOOPS EXTRACTS:

WATER SOLUBLE concentration (mg/plate)	BACTERIAL STRAINS TA 98	TA 100	TA 1537	ORGANIC SOLUBLE concentration (mg/plate)	BACTERIAL STRAINS TA 98	TA 100	TA 1537
0.53	-	236	20	0.27	34	216	11
1.50	43	272	-	0.58	40	187	13
2.47	41	253	-	1.74	36	118	7
5.04	41	268	-	2.90	40	24	6
7.50	-	228	-				

PRINCE GEORGE EXTRACTS:

WATER SOLUBLE concentration (mg/plate)	BACTERIAL STRAINS TA 98	TA 100	TA 1537	ORGANIC SOLUBLE concentration (mg/plate)	BACTERIAL STRAINS TA 98	TA 100	TA 1537
0.52	19	196	9	0.33	24	234	22
1.35	33	204	10	0.69	37	214	16
2.70	-	241	-	1.98	47	-	15
4.90	35	194	-	3.30	38	76	6

1. values represent mean of duplicated plates.

of 2,4,5-trichlorophenoxyacetic acid to beef cattle and Holstein dairy cows (Jensen *et al.*, 1981; Jensen and Hummel, 1982).

Although known toxic compounds were identified in the PFW samples in this study, no resin acids, chlorinated guaiacols or TCDFs were measured in the liver samples after the 63 days. The respective detection limits for these compounds were 400 ppb, 10 ppb and 0.2-0.5 ppb. Since microbial degradation of resin acids in pulp mill effluents occurs (Nestmann *et al.*, 1979), possibly the rumen microflora degraded any resin acids, thus bioaccumulation did not occur. However, the absence of dioxins in the liver samples is contrary to former studies (Jensen and Hummel, 1982). Traces of

dioxins in liver tissue were reported in cattle fed 0.024 µg 2,3,7,8-TCDD for 28 days (Jensen and Hammel, 1982). In this study beef cattle were exposed to 13.63 µg TCDF for 63 days. The differences between the two studies may be attributed to the lower dioxin detection limits in Jensen and Hummel's study. Also, the liver biopsy technique used in this study may not have permitted adequate sampling compared to the slaughter technique used by Jensen and Hummel (1982).

In summary, the results of this study show that potentially toxic chemical compounds are present in PFW and that the concentrations of these compounds differ between pulp mills. The differences in PFW composition from the two kraft mills reflect varying wood species, pulp washing practices and bleaching operations. Chemical characterization of each mill's PFW is fundamental to determine the suitability of PFW as a ruminant feed. Toxicological assessments of the chemical constituents in PFW including their bioactive properties are also recommended. Despite the identification of potentially toxic compounds in the PFW, no chlorinated phenols could be detected in the livers of beef animals exposed to this contaminated feed. These results raise questions concerning the biological significance of trace levels of contaminants in PFW and the safety of feeding this material to meat or dairy producing animals. Before reaching any conclusions concerning the safety of PFW as a feed, further studies on the bioavailability and tissue accumulation of chlorinated organics in the ruminant are required.

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