ENVIRONMENT CANADA

Forestry Service

PROJECT REPORT 11-4

ISOLATION OF TOXIC CONSTITUENTS OF KRAFT PULP MILL EFFLUENTS

B.C. Research

Progress to March 31, 1973

PULP AND PAPER POLLUTION ABATEMENT

A research program sponsored by the
Department of the Environment
in cooperation with the
Canadian Pulp and Paper Industry.

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FOREWORD

This report covers work done in the fiscal year 1972/73 on a project sponsored by the Cooperative Pollution Abatement Research program of the federal government and the Canadian pulp and paper industry.

The program was first announced in August, 1970 by the Minister of Fisheries and Forestry and the Minister of Energy, Mines and Resources, to provide for the funding of research contracts aimed at reducing water pollution from Canadian pulp and paper operations. Various elements of these Departments were combined with the formation in 1971 of the Department of the Environment, which assumed the responsibility for the operation and funding of the program. In November, 1971 the Minister of the Environment announced that the program would be expanded in the next fiscal year to include pulp and paper air pollution abatement research.

The program administration and Secretariat are provided by the Canadian Forestry Service, Department of the Environment. Development and guidance of the program are the responsibility of a joint government-industry committee known as the Committee on Pollution Abatement Research (CPAR). The members represent the Department of the Environment, The Department of Industry, Trade and Commerce, the Canadian Pulp and Paper Association, the Pulp and Paper Research Institute of Canada and the pulp and paper companies in eastern and western Canada.

The Committee plans the program, assesses priorities, reviews progress and advises on the allocation of funds and awarding of contracts for research proposals from pulp and paper companies and any other recognized research institutions. Based on the Committee's recommendations, the federal government enters into contract agreements with the organizations concerned for the conduct of approved projects.

In the fiscal year 1970/71 funds in an amount up to $500,000 were authorized to finance approved water pollution abatement projects. From 1971/72 through 1975/76 up to $1,000,000 will be available each year for water pollution research provided the pulp and paper industry's own annual expenditures for this type of work are increased by a like amount over the 1970 expenditures. For the fiscal year 1972/73 funds in an amount up to $200,000 became available on a similar basis for the support of approved pulp and paper air pollution abatement research projects. The same amount may be provided annually thereafter, through the fiscal year 1975/76.

CPAR PROJECT NO. 11

To: Canadian Forestry Service
Department of the Environment

ANNUAL REPORT FOR THE YEAR ENDING MARCH 31, 1973

ON

ISOLATION OF THE TOXIC CONSTITUENTS OF KRAFT PULP MILL EFFLUENTS

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A. BACKGROUND

An extensive survey of the toxicity exhibited to fish by the major process streams from seven British Columbia bleached kraft mills has been carried out by Howard and Walden (1971). They showed that unbleached whitewater (UWW), the main effluent from the pulping section, was frequently the most toxic stream. Bioassays were conducted on samples that had been filtered, air-stripped and neutralized in order to eliminate the toxicity due to volatile sulphides and mercaptans (Haydu et al 1952), and also that caused by abnormal pH (Howard and Walden 1965). Thus, the considerable toxicity shown by many UWW samples was the result of dissolved constituents in the effluent.

The primary objective of the present study was to isolate and identify all the non-volatile toxic constituents in UWW and to assess their individual contributions to the overall toxicity of the effluent. A secondary objective of the project was to provide a means for monitoring the toxicants and, if possible, to suggest methods for their removal or abatement.

B. PERSONNEL

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C. SUMMARY

1. All the non-volatile toxic constituents were identified in UWW from a British Columbia coastal kfaft mill pulping Douglas fir and western hemlock. Material and toxicity balances were maintained at all stages of a chemical fractionation procedure leading to isolation of the toxic factors.

2. The results have been published in a technical paper in the Journal of the Fisheries Research Board of Canada (Leach and Thakore 1973).

3. Resin acid soaps accounted for 82% of the toxicity of the UWW. The remaining 18% was caused by the presence of unsaturated fatty acid soaps.

4. The following resin acid soaps were present in the UWW:

isopimarate	5.6 mg/litre	(55%)
abietate	5.7 mg/litre	(22%)
dehydroabietate	2.2 mg/litre	(5%)
sandaracopimarate	0.6 mg/litre	(0%)

Figures in brackets refer to individual toxic contributions.

5. The fatty acid fraction included the following constituents:

C_{16} monounsaturated, palmitoleate	1.0 mg/litre
C_{18} monounsaturated, oleate	1.5 mg/litre
C_{18} di-unsaturated, linoleate	3.6 mg/litre
C_{18} tri-unsaturated, linolenate	1.1 mg/litre
C_{12} saturated, laurate	trace
C_{14} saturated, myristate	trace
C_{16} saturated, palmitate	1.9 mg/litre
C_{18} saturated, stearate	1.0 mg/litre
C_{20} saturated, arachidate	0.8 mg/litre
C_{22} saturated, behenate	trace

Only the unsaturated fatty acid soaps contributed to UWW toxicity. The saturated fatty acid soaps were not toxic at 20 mg/litre.

6. Confirmation that a toxicity balance had been maintained throughout the fractionation procedure was obtained by preparing a synthetic solution containing only the toxic constituents in the concentrations that they were found in the UWW. The synthetic mixture had the same toxicity over a wide range of concentrations as the original UWW sample.

7. Information was obtained on the relative toxicities of certain resin and fatty acid soaps. Pimaric-type resin acid soaps tended to be more toxic than abietic-type soaps. Fatty acid soaps were less toxic than resin acid soaps over the range of concentrations tested. The toxicity of fatty acid soaps appeared to increase with the degree of molecular unsaturation.

8. The toxic constituents of UWW are all derived from extractives in the wood chips used in the kraft cook. In view of their low concentrations, there is no chemical basis for supposing a relationship between BOD_5 or TOC and toxicity for this effluent.

9. A sensitive, selective gas chromatographic procedure was developed for analysis of resin and fatty acids in pulp mill effluents. The procedure has been successfully tested on a variety of process streams. Individual resin and fatty acid concentrations can be measured to a detection limit of approximately 0.01 mg/litre in most cases. The analysis requires only 3-4 hr to complete. Several can be done simultaneously.

10. Thirteen UWW samples from eleven widely dispersed kraft mills have been analyzed for resin acid soaps and fatty acid soaps. Resin acid concentrations ranged from 2.3 to 45.8 mg/litre. The most common resin acids were isopimaric, abietic and dehydroabietic. Lesser amounts of pimaric, sandaracopimaric, levopimaric and neoabietic acids were detected on occasions.

C_{16} and C_{18} unsaturated fatty acid concentrations were between. 0.5 and 15.7 mg/litre in UWW samples tested.

11. UWW from the pulping of jackpine or spruce contained the highest concentration of resin acid soaps. Detailed comparisons between the effects of various wood furnishes on resin and fatty acids in UWW have not been possible, however, because of the limited number of samples and differences in brownstock washing practices.

D. MATERIALS AND METHODS

1. General

Amberlite XAD-2 macroreticular resin (Rohm and Haas Company) was extracted before use with ether, methanol and water to eliminate soluble, low molecular-weight material. Unbleached whitewater was sparged with nitrogen to remove oxygen and volatile constituents prior to passage at 2C over the resin. Solvents were removed rapidly from separated chemical fractions by evaporation under reduced pressure at temperatures below 40C to minimize isomerisation of thermodynamically unstable species. Dried isolates were stored at -10C.

Silica gel (J.T. Baker) and silica gel impregnated with 25% silver nitrate (Applied Science Laboratories Inc., "Adsorbosil CABN")were used for column chromatography.

Separated fractions were monitored by gas chromatography (GC) and thin layer chromatography (TLC). GC analyses were carried out on Perkin-Elmer Model 881 or F-11 temperature-programmed instruments equipped with flame ionisation detectors. Three stainless steel columns were used. Most work was done on a 6 ft. x 1/8 in. column containing 10% diethyleneglycol succinate (DEGS) on 60-80 Chromosorb W. When a non-polar support was required, 15% SE30 on Chromosorb W was used. A third column of intermediate polarity containing 3% QF-1 on 60-80 Gas Chrom Q was employed occasionally.

Individual compounds were identified initially by combination GC-mass spectrometry (MS). Eluates from a Varian Associates 1600 Aerograph GC instrument containing a DEGS column were passed directly into a Hitachi Perkin-Elmer RMU-6E mass spectrometer. Full structural characterization was obtained from infrared (IR), ultraviolet (UV) and nuclear magnetic resonance (NMR) spectra.

Pure samples of fatty acids were purchased from Applied Science Laboratories Inc. Resin acids were either separated from oleoresin as described later or donated by other laboratories.

2. Bioassays

Toxicities of neutralized effluents and isolates were compared in static bioassays at 11C using underyearling coho salmon, Oncorhynchus kisutch, mean body weight 1.2g. The volume of bioassay solution was limited by the amounts of materials available, to 3 litres in the early stages of the separation and later to 6 litres when the fish were larger. Since fish density was higher than recommended for static bioassays (Sprague 1969), the data were used for intercomparison only. Median survival times (MST) of ten fish were measured up to a maximum of 96 hr in several concentrations of each solution. Solutions which permitted

100% survival of fish for 96 hr were considered to be non-toxic. Dilutions were made with dechlorinated water (pH 6.4, EDTA hardness 6 mg $CaCO_3$/ litre) and solutions were aerated minimally to maintain dissolved oxygen at saturation level. Where aliquots from etherial solutions of chemical fractions were used in bioassays, the control solution contained ether at the maximum concentration occurring in the test solutions. The normal hyperbolic relationship between toxicant concentration and MST was expressed in a linear form using logarithmic plots (Sprague 1969). The log concentration - log MST graph for the original UWW was derived from the mean MST values of four replicate bioassays at a number of dilutions of a composite sample representing effluent processed through the XAD-2 resin. To ensure maintenance of a toxicity balance at each stage in the fractionation procedure, individual and recombined fractions were dissolved in alkali, neutralized and tested by bioassays. Toxic contributions of chemically discrete fractions were found by graphical interpolation.

3. Large-Scale Fractionation and Isolation of the Toxic Constituents of Unbleached Whitewater

Unbleached whitewater (850 litres) was collected from the screening and deckering area of a B.C. coastal bleached-kraft mill during 2 hr of normal operation. At the time of collection, a mixture of Douglas fir and western hemlock was being cooked, and Kamyr and batch digesters were in use. The effluent was returned to the laboratory and allowed to stand at 2C overnight to permit settling of pulp fibres. Material remaining in suspension was removed by filtration through rayon cloth. The effluent was then passed continuously during an 84 hr period through Amberlite XAD-2 resin (14 litres) contained in seven glass columns (4 ft x 2 3/4 in). Figure 1 shows a diagram of the experimental arrangement.

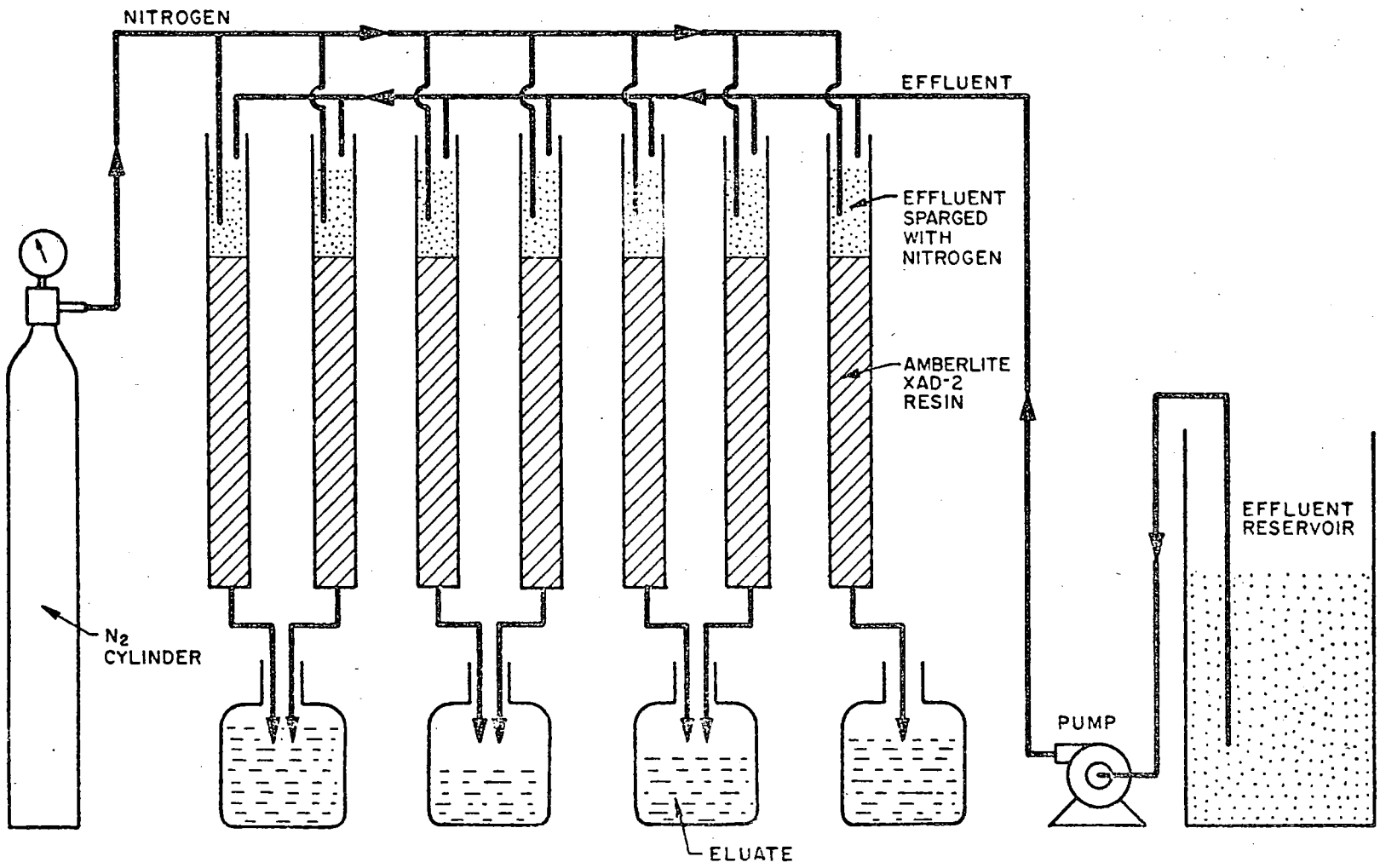
3-Litre aliquots of eluate were taken for toxicity testing from every 60 litres of effluent passed through the columns.

Material adsorbed by the resin was removed by passage of ether (70 litres). Solvents were evaporated from both fractions and the residues dried in vacuo over KOH pellets. The ether fraction gave a toxic, dark-brown gum (61.83 g). The methanol residue was not toxic and was not examined further.

The ether extract (59.36 g) was chromatographed on a column of 40-140 mesh silica gel (630 g). Successive elutions with petroleum ether (BP 30-60C) and methanol gave a colourless, non-toxic oil (32.13 g) and a toxic, semi-crystalline brown oil (26.68 g), respectively. GC and TLC analysis of the brown oil, after methylation with diazomethane, indicated the presence of fatty acids and resin acids.

Figure 1

EXPERIMENTAL ARRANGEMENT FOR PROCESSING U W W



The brown oil (22.14 g) was divided into three portions and the fatty acids in each portion were selectively esterified using a modification of the method of Sheers and Stevenson (1960). In a typical reaction, the extract (7.91 g.) was dissolved in methanol (200 ml), 25% H_2SO_4 (10 ml) added and the solution stirred at room temperature for 16 hr. Methanol was evaporated, the residue dissolved in ether (300 ml) and the unesterified resin acids extracted into 10% NaOH solution (3 x 100 ml). The aqueous layer was separated, acidified and extracted with diethyl ether (4 x 100 ml). The fraction containing free resin acids was recovered on removal of solvent. Total yield was 14.68 g. The fatty acid methyl esters, which remained in ether, were saponified with 40% NaOH solution and free fatty acids (total yield 8.29 g) were recovered by the same procedure as that used for resin acids.

The resin acid fraction (4.03 g) was decolourized by chromatography on silica gel (469 g). Resin acids (2.73 g) were eluted gradually in chloroform followed by coloured components (0.92 g) in methanol. The resin acids (1.00 g) were fractionated on a column of silica gel impregnated with 25% silver nitrate by a modification of the technique of Norin and Westfelt (1963). Slow elution with chloroform gave a mixture of abietic-type acids (0.56 g) followed by a mixture of pimaric-type acids (0.44 g). The two fractions were chromatographed again using gradient elution from 50/50 petroleum ether-benzene to 100% benzene. Abietic-type acids (0.40 g) were separated into dehydroabietic acid (0.11 g) and abietic acid (0.29 g); pimaric-type acids (0.32 g) were separated into isopimaric acid (0.29 g) and sandaracopimaric acid (0.03 g). Resin acid composition was checked by GC before and after silver nitrate-silica gel chromatography to ensure that no chemical modification had occurred. The identity and purity of each compound was confirmed by comparison of spectroscopic data with literature values (Zinkel et al 1971) and with IR, UV and NMR spectra obtained for authentic samples.

The fatty acid fraction was methylated with diazomethane and analyzed by GC (DEGS column, programmed from 50-150C at 12C/min, N_2 flow 30 ml/min) using behenic acid as an internal standard. The identity of each fatty acid methyl ester was confirmed by combination GC-MS using pure samples for comparison.

4. Small-Scale Fractionation of the Toxic Constituents of UWW

A batch of UWW from a B.C. interior mill that was pulping a mixture of Douglas fir, lodgepole pine, spruce and alpine fir and one from an Ontario mill operating on a wood furnish of jackpine and dense hardwood were separated into toxic fractions on a smaller scale. Each effluent (10 litres) was passed through a glass column containing Amberlite XAD-2 resin (2 litres). Eluate from the resin was tested for toxicity by a bioassay at 100% concentration. The resin was then eluted with 10:1 ether-methanol (5 litres), solvent was evaporated and the yellow, oily residue (2.76 g) diluted with ether to 100 ml in a volumetric flask. Aliquots of the ether solution were dissolved in water for bioassays. Analyses for individual resin and fatty acids were carried out on the ether extract using the procedure described in Section 6. Solvent was removed from the remaining portion (50 ml, equivalent to 5 litres UWW), the residue was dissolved in methanol (50 ml), 25% H_2SO_4 (0.5 ml) added and the mixture stirred for 16 hr at room temperature. Methanol was then evaporated, the residue dissolved in ether and resin acids extracted with 10% NaOH solution (3 x 50 ml). Free resin acids were recovered as before. Fatty acid methyl esters in the ether layer were saponified with 40% NaOH and the free fatty acid fraction isolated in the usual way. Bioassays and GC analyses were carried out on the resin acid and fatty acid fractions separately.

5. Preparation of Pure Resin Acid Standards

Procedures are given in the literature for the isolation of pure resin acids from oleoresin or from crude mixtures of the acids. However, because of inconsistent quality in the starting materials, strict adherence to the literature techniques often did not result in good yields of the desired products. It is not practical to list all the modifications to literature procedures that were used in preparing samples of resin acids for use in this project, since the same variations in technique may not be applicable to starting material from other sources. Consequently, for each resin acid preparation a reference is given which contains a basic experimental procedure.

(a) Abietic Acid

Prepared from technical grade abietic acid (100 g) using the procedure outlined in Organic Syntheses (1952). Yield 13.6 g.

(b) Dehydroabietic Acid

Isolated from technical grade dehydroabietic acid (100 g) using a modified procedure of Halbrook and Lawrence (1966). Yield 6 g.

(c) Levopimaric Acid

Separated from southern pine oleoresin using a procedure based on that described by Summers et al. (1963)

(d) Isopimaric and Pimaric Acids

Starting from southern pine oleoresin (600 g), a concentrated resin acid mixture (44 g) was separated by modifying the procedure of Baldwin et al. (1958). A fraction rich in pimaric-type acids was then isolated and small samples of pure isopimaric and pimaric acids were obtained by column chromatography.

6. GC Analysis of Resin and Fatty Acids

UWW (1 litre) was adjusted to pH10 and passed through XAD-2 resin (75 ml) in a 1 ft glass chromatography column at a rate of approximately 10 ml/min. The adsorbed materials were eluted in 10:1 ether-methanol (1 litre) and the ether layer was then separated from the aqueous layer, which was present due to residual effluent in the resin. After drying ($MgSO_4$), the ether solution was filtered, and the solvent then removed. The residue was transferred to a glass vial using ether (5 ml), and heptadecanoic acid solution (5 ml of 255 mg/litre solution) added as an internal standard. Solvent was evaporated in a nitrogen stream and a solution of diazomethane in ether added to the residue to convert resin and fatty acids to their methyl esters. The solution was concentrated by evaporating the ether in a stream of nitrogen. A sample (0.5 - 2 ml) was then analyzed by gas chromatography using a DEGS column (N_2 flow rate 85 ml/min; 10 min at 150C followed by temperature programming from 150 - 175C at 2C/min).

Response factors and retention times for individual resin and fatty acids were compared regularly with the internal standard by injecting solutions containing known amounts of the standard and the reference acid.

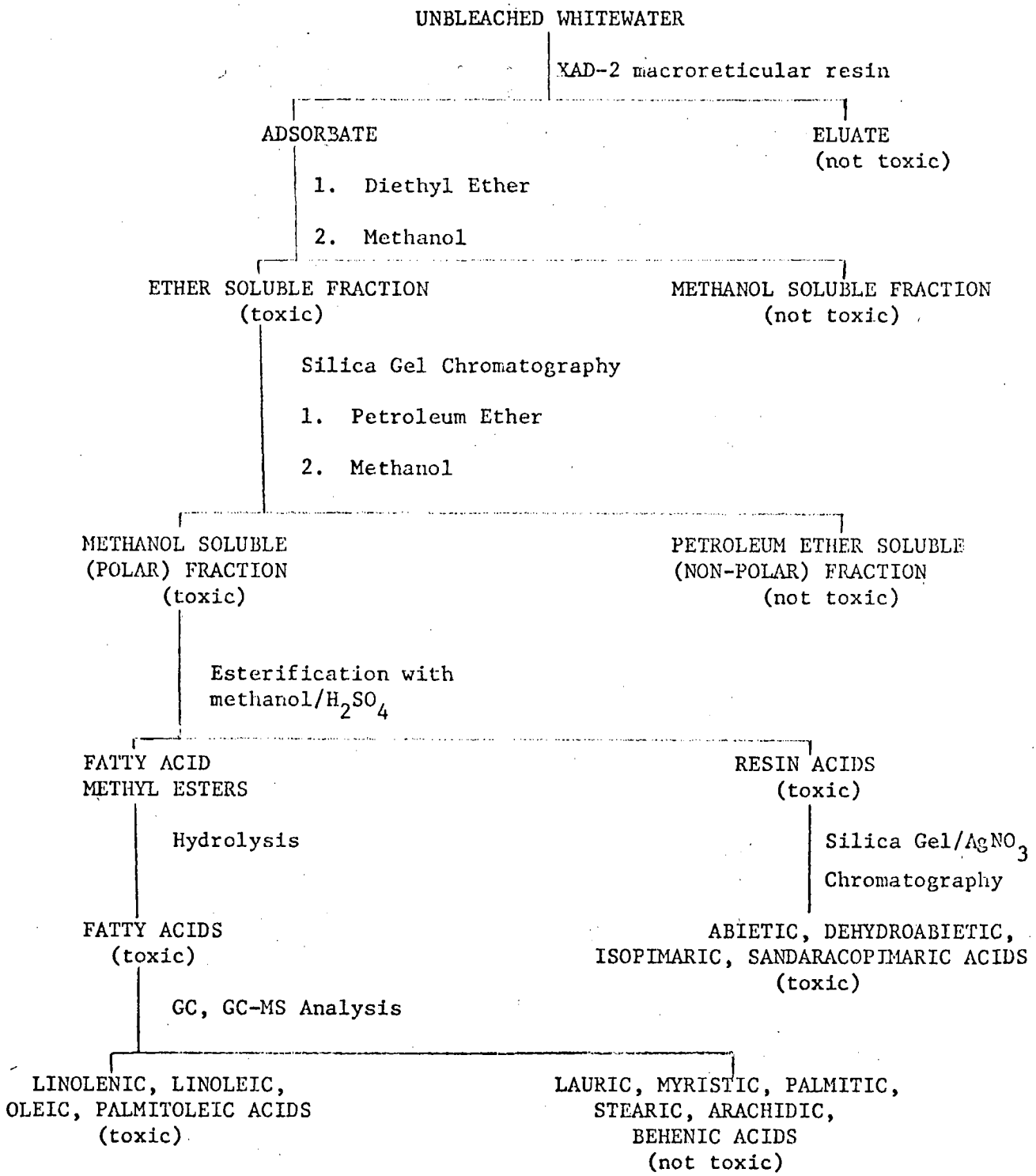
The area of each peak of interest on the GC trace was measured by a planimeter and resin and fatty acid concentrations calculated from the formula:

$$Wa = \frac{Aa}{As} \times Ws \times f$$

Where f is the response factor ($= \frac{Wa}{Ws} \cdot \frac{As}{Aa}$, calculated from standards)

and Wa = weight of resin or fatty acid in sample
Ws = weight of added heptadecanoic acid standard
Aa = area of resin or fatty acid peak
As = area of heptadecanoic acid peak.

FIGURE 2



FRACTIONATION SCHEME FOR UWW TOXIC CONSTITUENTS

E. RESULTS

1. Large-scale Separation of the Toxic Constituents of UWW

All the non-volatile constituents toxic to juvenile coho salmon were identified in UWW collected from a kraft mill that was pulping a mixture of Douglas fir and western hemlock. Material and toxicity balances were fully accounted for throughout the separation procedure. Figure 2 shows the complete fractionation scheme leading to isolation of the toxic constituents.

In the first stage of the separation procedure, the toxic materials were adsorbed from UWW by Amberlite XAD-2 resin. Though there was no detectable decrease in the colour of the effluent after passage through the resin, all test fish survived in regularly sampled aliquots of the eluate. This fact suggested that highly-coloured lignin fragments were unlikely to be important toxic factors. Bioassays showed that the toxic materials were completely recovered on washing the resin with ether. The graph of log concentration vs. log MST for the ether extract was identical with that for the original effluent. Subsequent elution of the resin with methanol gave a dark brown solution, but its constituents were not toxic.

Chromatography of the ether-soluble material on silica gel permitted the separation of non-polar materials from polar compounds on the basis of their solubilities in petroleum ether and methanol. The non-polar fraction was not toxic. The polar fraction from the chromatography had toxicity-concentration characteristics identical with the starting UWW and the ether extract of the XAD-2 resin.

At this stage, an indication of the chemical identity of the components in the methanol fraction was needed in order to pursue further separations. From GC and TLC analysis and IR and NMR spectroscopy it was concluded that the polar fraction contained a variety of resin acids and also long-chain fatty acids. Resin acids are resistant to esterification because of steric hindrance around the carboxyl group. By using mild conditions, therefore, it was possible to selectively esterify the fatty acids without affecting the resin acids, and thereby to effect separation of the two. GC analysis confirmed that no isomerisation or losses of resin acids were suffered during the separation.

Coloured material was removed from the resin acid mixture by column chromatography and the pure resin acid and fatty acid fractions were then tested separately for toxicity by bioassays.

Resin acids, present in the UWW as their sodium salts at a concentration of 14.1 mg/litre, accounted for 82% of the UWW toxicity. The remaining 18% of the toxicity was due to the fatty acid fraction, present in UWW at 10.9 mg/litre.

FIGURE 3

BIOASSAY DATA FOR UNBLEACHED WHITEWATER AND ISOLATED TOXIC FRACTIONS

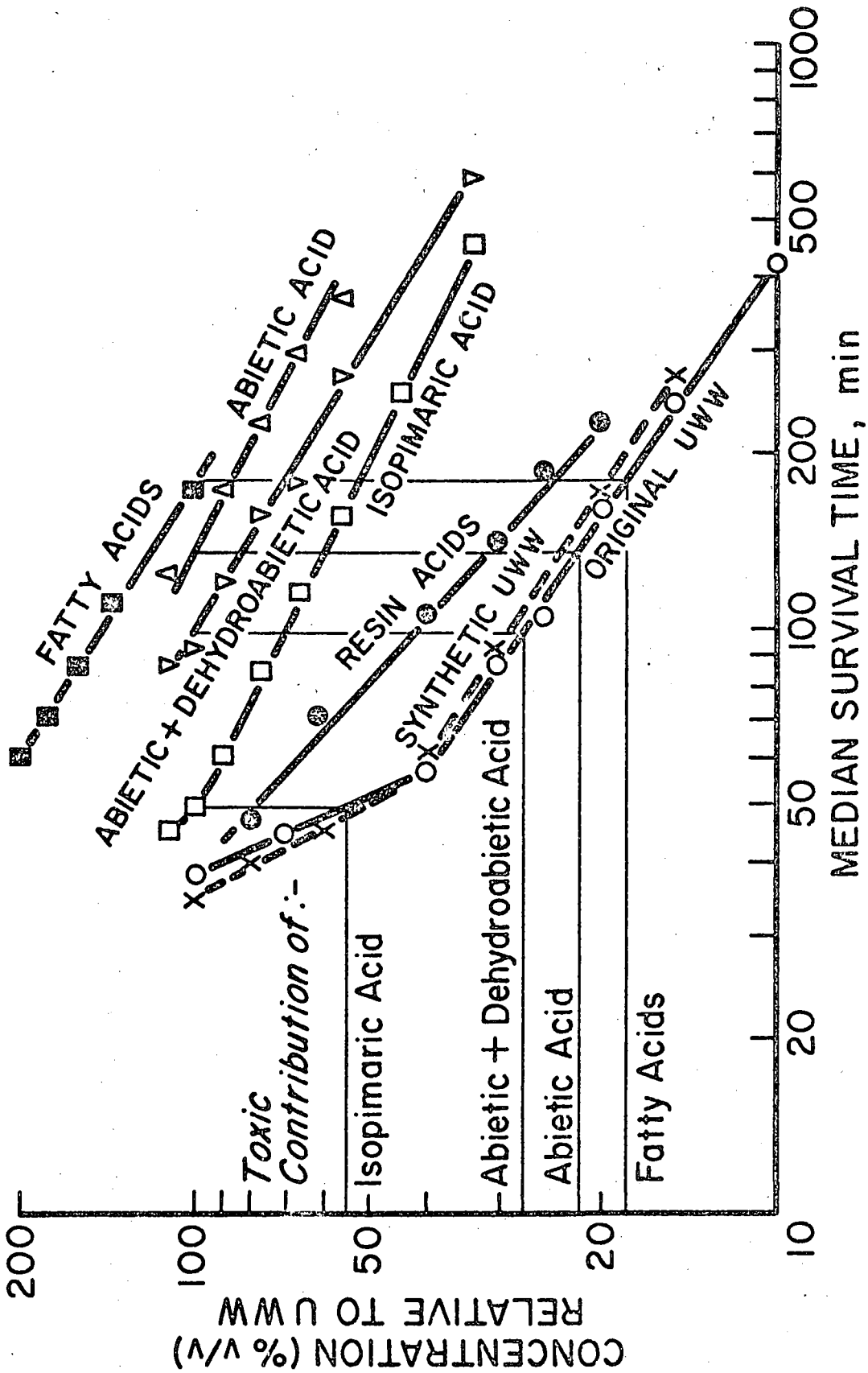
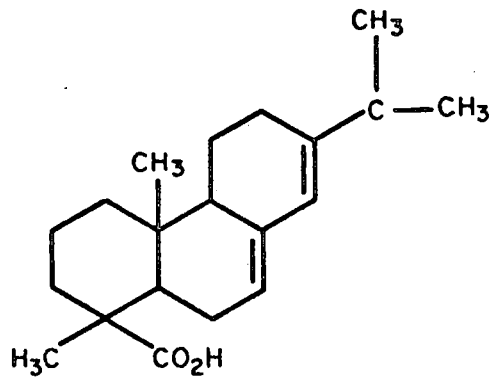
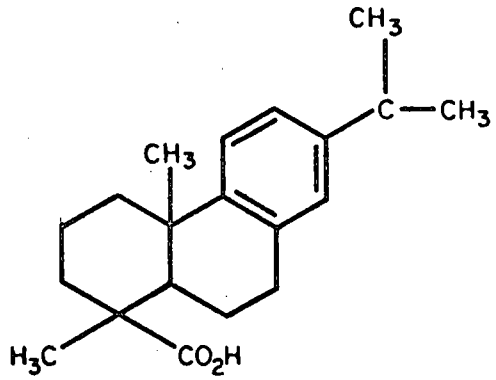


Figure 4

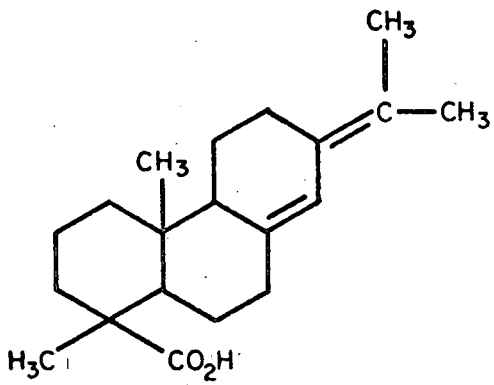
STRUCTURAL FORMULAE OF RESIN ACIDS



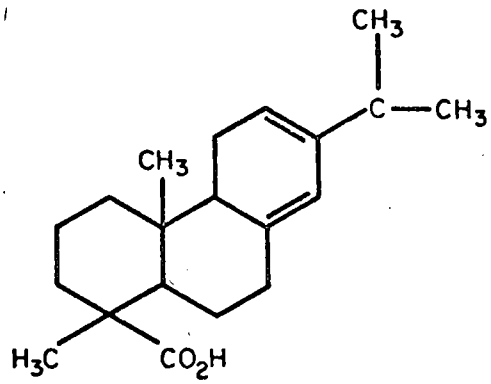
ABIETIC ACID



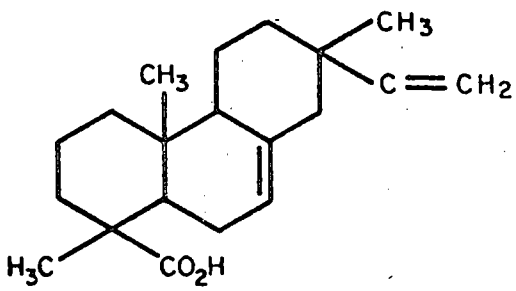
DEHYDROABIETIC ACID



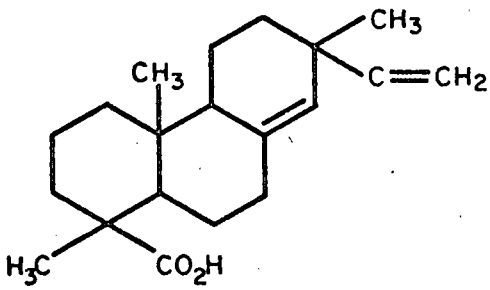
NEOABIETIC ACID



LEVOPIMARIC ACID



ISOPIMARIC ACID



SANDARACOPIMARIC ACID

Individual resin acids were obtained after chromatographing the mixture twice on silica gel impregnated with silver nitrate. After the first pass, the resin acids were separated into abietic and pimaric types. Both fractions were re-chromatographed using different solvents and four, pure resin acids were obtained. Bioassays were done on the sodium salt of each resin acid and the individual toxic contributions to UWW toxicity were established by graphical interpolation.

Sodium isopimarate was present in the UWW at 5.6 mg/litre, and accounted for 55% of the toxicity (see Figure 3). Sodium abietate was found at a concentration of 5.7 mg/litre and was responsible for 22% of the UWW toxicity. Sodium dehydroabietate did not kill fish when tested at the concentration in which it was isolated from UWW (2.2 mg/litre). However, the fish were distressed and showed "cough" symptoms typical of exposure to high sub-lethal levels of pulp mill effluent (Schaumburg et al, 1967). The abietate-dehydroabietate mixture isolated from UWW accounted for 27% of the toxicity (see Figure 3) and the toxic contribution of sodium dehydroabietate was thus established by difference as 5%. The fourth resin acid isolated from the UWW was sandaracopimaric acid. At the concentration found in the UWW, viz 0.6 mg/litre, it was not toxic, and had no measurable effect on the toxicity of the isopimarate fraction.

Structural formulae of the four isolated resin acids together with two other common resin acids, are shown in Figure 4.

As confirmation that all resin acid soaps in the UWW had been identified, a synthetic mixture was made using the concentrations at which they were found in UWW. Bioassays showed that the synthetic mixture had the same concentration - toxicity graph as the isolated resin acid fraction.

The fatty acid fraction was too complex to isolate sufficient quantities of the individual constituents for bioassays. GC analysis revealed at least 24 constituents and GC-MS was used to identify the major ones. Concentrations of the individual fatty acid soaps were calculated from quantitative GC analysis of the mixture. Table 1 lists the fatty acid soaps present in the UWW.

TABLE 1

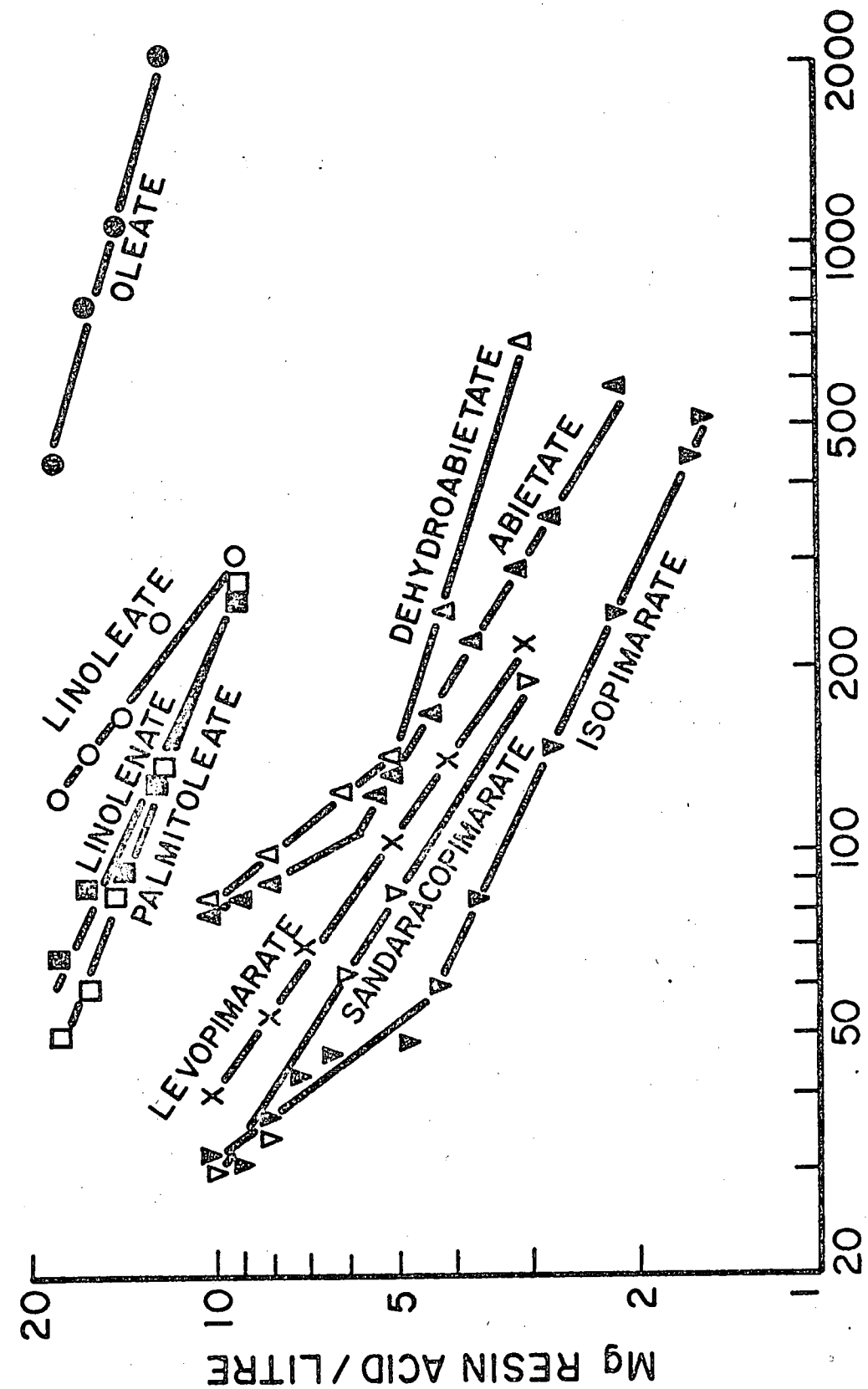
FATTY ACID SOAPS IN UWW

CARBON NUMBER	ACID	CONCENTRATION IN UWW (mg/litre)
C_{12}	Laurate	trace
C_{14}	Myristate	trace
C_{16}	Palmitate	1.9
C_{16}^{9}	Palmitoleate	1.0
C_{18}	Stearate	1.0
C_{18}	Oleate	1.5
$C_{18}^{9,12}$	Linoleate	3.6
$C_{18}^{9,12,15}$	Linolenate	1.1
C_{20}	Arachidate	0.8

Bioassays were done on the sodium salts of all the fatty acids in Table 1 at concentrations up to 20 mg/litre. Only the unsaturated fatty acids were toxic, though individually they did not kill fish at the concentrations in which they were found in UWW. However, a solution containing palmitoleate, oleate, linoleate and linolenate, at the concentrations shown in Table 1, gave a concentration-toxicity graph which was indistinguishable from the one produced by the fatty acid fraction isolated from UWW. The toxicity of the fatty acid soaps decreased rapidly when diluted below their concentration in 100% UWW. In a solution containing 90% of the UWW concentration of the fatty acid sodium salts, fish were disoriented but were not killed.

FIGURE 5

BIOASSAY DATA FOR VARIOUS RESIN AND FATTY ACID SODIUM SALTS



Traces of methyl abietate and methyl isopimarate were detected by GC in the fatty acid fraction. They were also shown to be present in the ether extract from XAD-2 resin, which indicates that they originate from the UWW. The methyl esters were not toxic when tested at 2-4 mg/litre, which is much greater than their concentration in UWW.

As a final demonstration that all the toxic factors in UWW were accounted for, a solution was made up containing the resin acid fraction plus individual, unsaturated fatty acids at the concentrations found in UWW. Bioassay results over a range of concentrations of the solution were within 2% of those obtained in the original effluent (Figure 3).

Figure 5 shows the relative toxicities of various resin and fatty acid sodium salts. For isopimarate, no mortality occurred at 1 mg/litre; for all other acids shown, fish survived in excess of 96 hr in solutions containing 1 mg/litre less than the lowest concentration plotted. Under the test conditions, therefore, lethal threshold concentrations for resin acid soaps were between 1 and 3 mg/litre and for fatty acid soaps, except oleic, the lethal thresholds were between 8 and 9 mg/litre. The data do not represent precise incipient LC50 values since the static bioassay conditions used may have permitted some detoxification of the solutions.

2. Fractionation of the Toxic Constituents in Other UWW Samples

(a) B.C. Interior Mill

The toxic constituents in an UWW sample from a B.C. interior mill pulping a softwood mixture were quantitatively adsorbed by XAD-2 resin. Bioassays showed that 95% of the effluent toxicity was recovered by extracting the adsorbed constituents from the resin with an ether-methanol mixture.

The 5% of toxicity which was not accounted for may have been the result of losing labile materials during processing, or may have been due to incomplete removal of the adsorbed toxic constituents from XAD-2 resin, or possibly because of experimental error in measurement of MST's. The ether-methanol extract was separated in the manner described previously into a resin acid fraction and a fatty acid fraction. Together, the two fractions accounted for all the toxicity in the extract, as demonstrated by the log concentration-log MST graphs shown in Figure 6. The fatty acid fraction, which was shown by GC analysis to contain 7.97 mg of C_{16} and C_{18} unsaturated fatty acids per litre UWW, was more toxic than the resin acid fraction. Total resin acid concentration was 7.22 mg/litre UWW and the mixture included sandaracopimaric, isopimaric, abietic and dehydroabietic acids. Excellent agreement was obtained between the quantitative resin and fatty acid analysis carried out on the ether-methanol extract of XAD-2 resin and analyses done on the separated resin and fatty acid fractions.

Figure 6

BIOASSAY DATA FOR TOXIC FRACTIONS IN U W W FROM A B. C. INTERIOR MILL

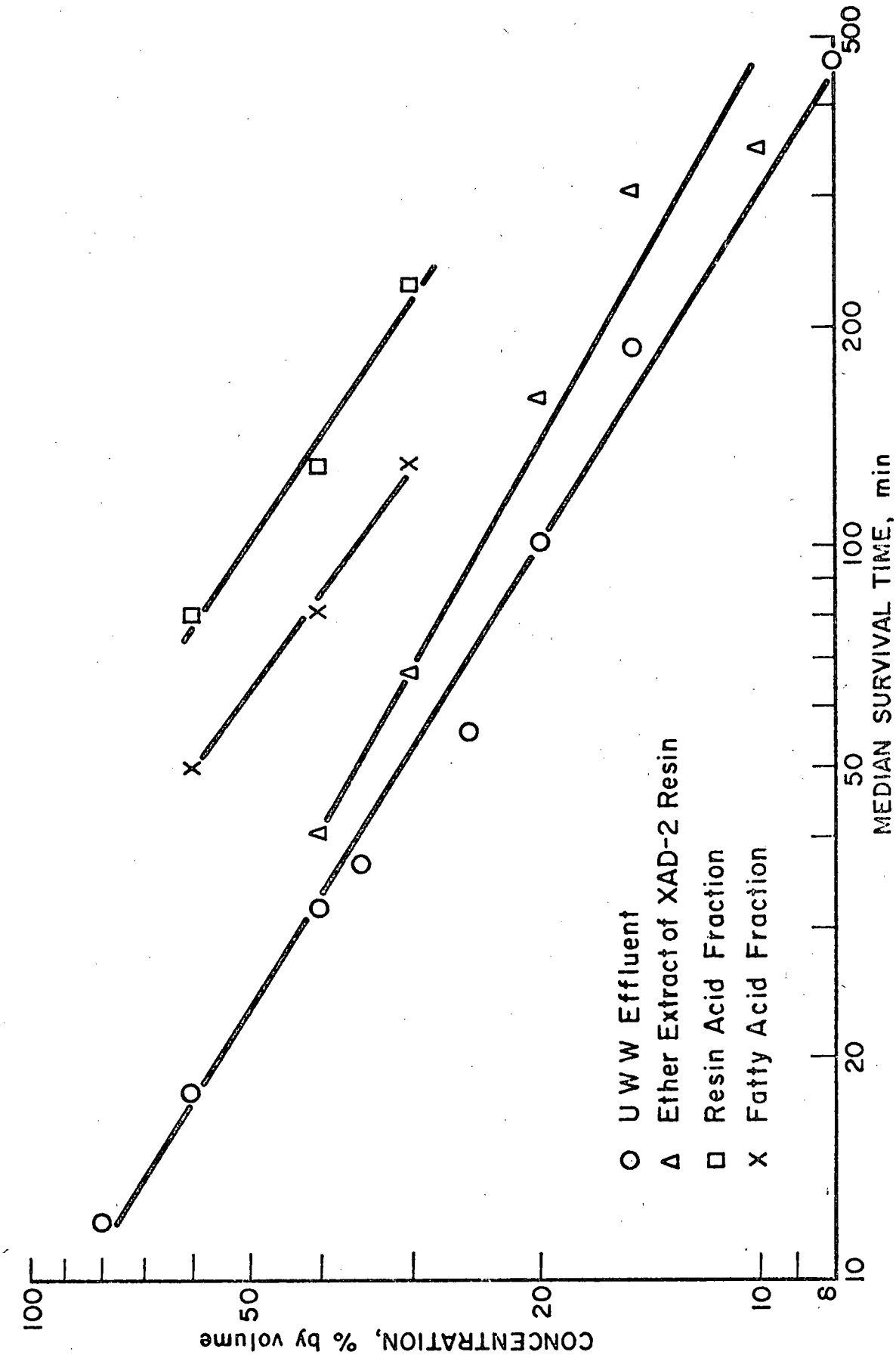


Figure 7

BIOASSAY DATA FOR TOXIC FRACTIONS IN U W W FROM AN ONTARIO MILL

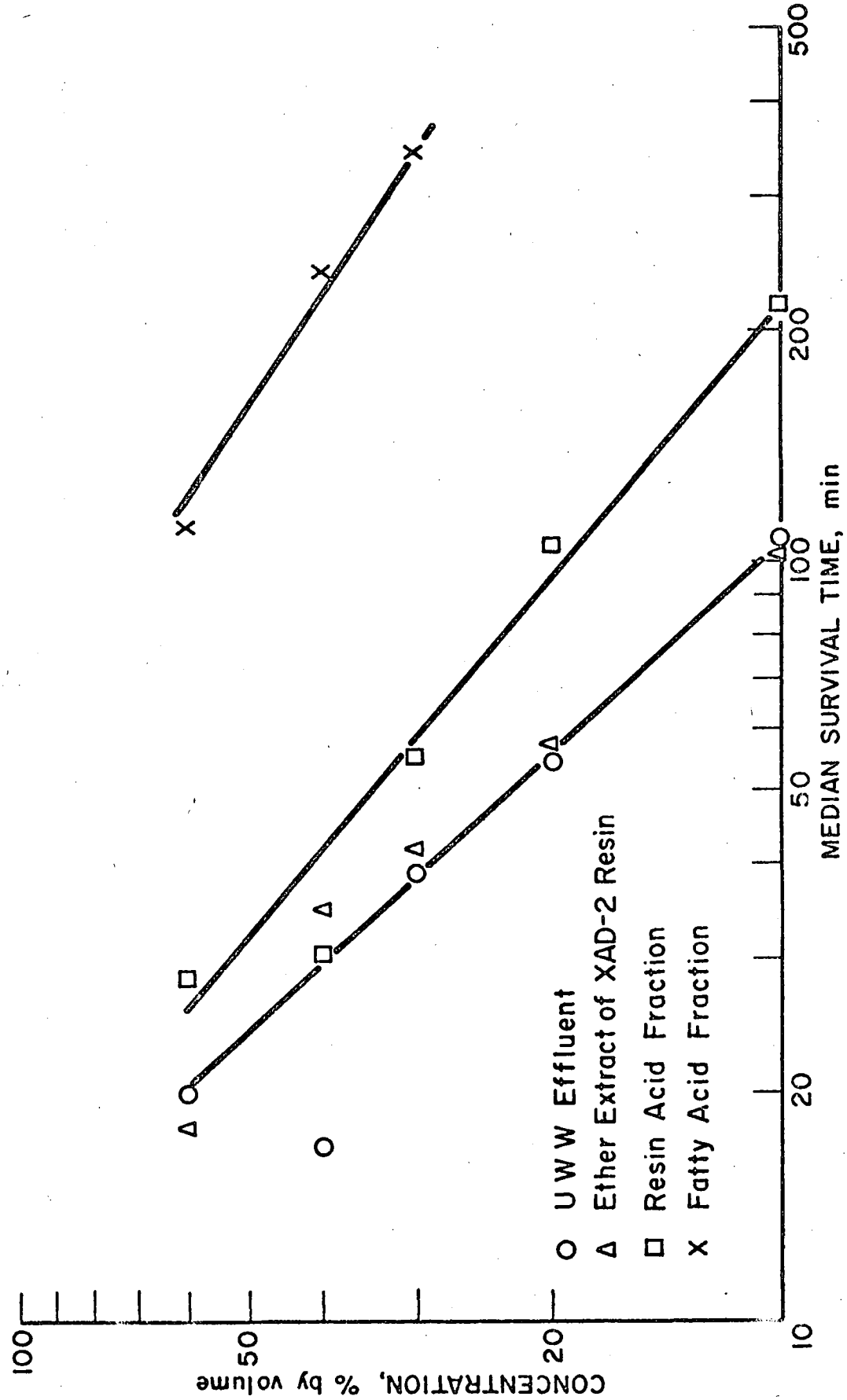


TABLE 2

RESIN ACID RECOVERY AS A FUNCTION OF pH

SAMPLE NO.	RESIN ACID	pH of SOLUTION	% RECOVERY	
			INDIVIDUAL ACIDS	MEAN
1	P A + D	10	100 107	104
2	P A + D	10	101 102	102
3	P A + D	10	100 100	100
4	P A + D	7	100 94	97
5	P A + D	7	100 101	100
6	P A + D	7	96 97	96
7	P A + D	7	90 90	90
8	P A + D	2	77 64	70
9	P A + D	2	69 54	62
10	P A + D	2	87 93	90
11	P A + D	2	53 50	52
12	P A + D	2	83 77	80

Key P = pimaric acid
A + D = abietic + dehydroabietic acids

(b) Ontario Mill

A batch of UWW from an Ontario mill that was pulping a mixture of softwood and hardwood was passed through XAD-2 resin and the toxic factors were completely adsorbed in the usual way to give a non-toxic eluate. Quantitative recovery of the adsorbed toxicants was obtained by elution with ether-methanol solution and additional separation by selective esterification led to the isolation of resin and fatty acid fractions. Soaps of the resin acids, pimaric, sandaracopimaric, isopimaric, abietic and dehydroabietic amounted to 32.3 mg/litre UWW and were the major contributors to the effluent toxicity (see Figure 7). Unsaturated C_{16} and C_{18} fatty acid soaps were present at a total concentration of 15.7 mg/litre and accounted for the remaining toxicity. Again, there was excellent agreement between concentrations obtained for the resin-fatty acid mixture and results of analyses on the separated fractions.

3. GC Analysis of Resin and Fatty Acids

(a) Development of the Procedure

Several variables were studied during the course of adapting the procedure for isolating the toxic constituents of UWW into a routine analytical method for these toxicants. They included pH, sample volume, volume of adsorbent resin, eluting solvent, choice of internal standard, GC conditions, and GC response factors for resin and fatty acids. A brief description is given below of the effects of each variable on the results of analyses for resin and fatty acids.

(i) pH

Standard solutions containing a mixture of pimaric, abietic, and dehydroabietic acids were made at pH 2,7 and 10. Recoveries of the resin acids were measured by GC after adsorption on to XAD-2 resin and subsequent desorption in 10:1 ether-methanol. Table 2 contains the results.

The percentage recovery of resin acids improved with increasing solution pH. At pH 10 essentially complete recovery was obtained for all samples tested. Recoveries in the range 90-100% were obtained for solutions processed at pH 7 and erratic recoveries of 52-90% were recorded for pH 2 solutions of the resin acids. The latter recoveries may be a consequence of the poor solubility of resin acids at pH 2.

(ii) Sample Volume

The required sample volume varied depending upon the nature of the effluent being analysed. For maxiumum accuracy, the optimum weight of resin acids in a sample was 5-10 mg. Effluent volumes of 100 ml - 1 litre generally permitted resin acid weights in this range to be obtained in the solution for GC analysis.

(iii) Volume of Adsorbent

When 75 ml XAD-2 resin was used as the adsorbent, recoveries for a standard solution containing 13 mg resin acids were excellent. On reducing the volume of adsorbing resin to 35 ml, approximately 10% loss of resin acids was suffered. For some effluent samples, 75 ml resin may be insufficient unless the effluent volume processed is reduced below 100 ml or the effluent is diluted.

A more polar resin, Amberlite XAD-7, was investigated but it presented no advantages over the well-tried XAD-2 resin.

(iv) Eluting Solvent

When ether alone was used as the solvent for eluting adsorbed material from XAD-2 resin, recoveries were approximately 5% low. Methanol gave excellent resin and fatty acid recoveries but led to problems in drying the eluate prior to solvent removal because of its miscibility with the residual effluent trapped in the resin. A compromise solution using a 10:1 ether-methanol mixture gave excellent recovery of adsorbed resin and fatty acids and no drying problems.

(v) Internal Standard

An internal standard was used in preference to an external standard for GC analysis because of the greater accuracy attainable. When an external standard is used, precisely measured microlitre volumes of sample and standard must be injected into the GC in successive runs under identical instrument conditions. In the case of internal standardization, the volume of solution injected is not critical and GC conditions are the same for sample and standard since they are run simultaneously.

For most effluent samples, heptadecanoic acid was a suitable internal standard, since is occurs only infrequently in nature (Nestler and Zinkel 1967). It was usually well resolved from neighbouring GC peaks. Pentadecamoic acid was investigated but the GC peak was often too close in retention time to other components. On occasions the entire fatty acid region of the GC trace was too complex to permit reliable measurement of the internal standard peak. In such cases, a resin acid, usually dehydroabietic or pimaric acid, was used as the internal standard.

The volume of internal standard solution added to the sample was such that the weight of standard approximately equalled the weight of the most abundant resin or fatty acid in the sample. These conditions gave the best accuracy in measurement of GC peak areas.

(vi) GC Conditions

Various stationary phases were examined for their ability to separate mixtures of C_{12} to C_{24} saturated and unsaturated fatty acids and the complete range of resin acids normally encountered in pulp and paper effluents.

Non-polar phases, such as SE-30, gave poor resolution of fatty acids, analysed as their methyl esters, and only moderate separation of resin acid methyl esters. The polar phases, DEGS and EGSS-X, gave good separations of fatty acid methyl esters and resin acid esters. DEGS columns were selected for regular use since they resolved pimarate from levopimarate and palustrate. On EGSS-X all three compounds have the same retention time. No stationary phase was found that could separate levopimarate from palustrate.

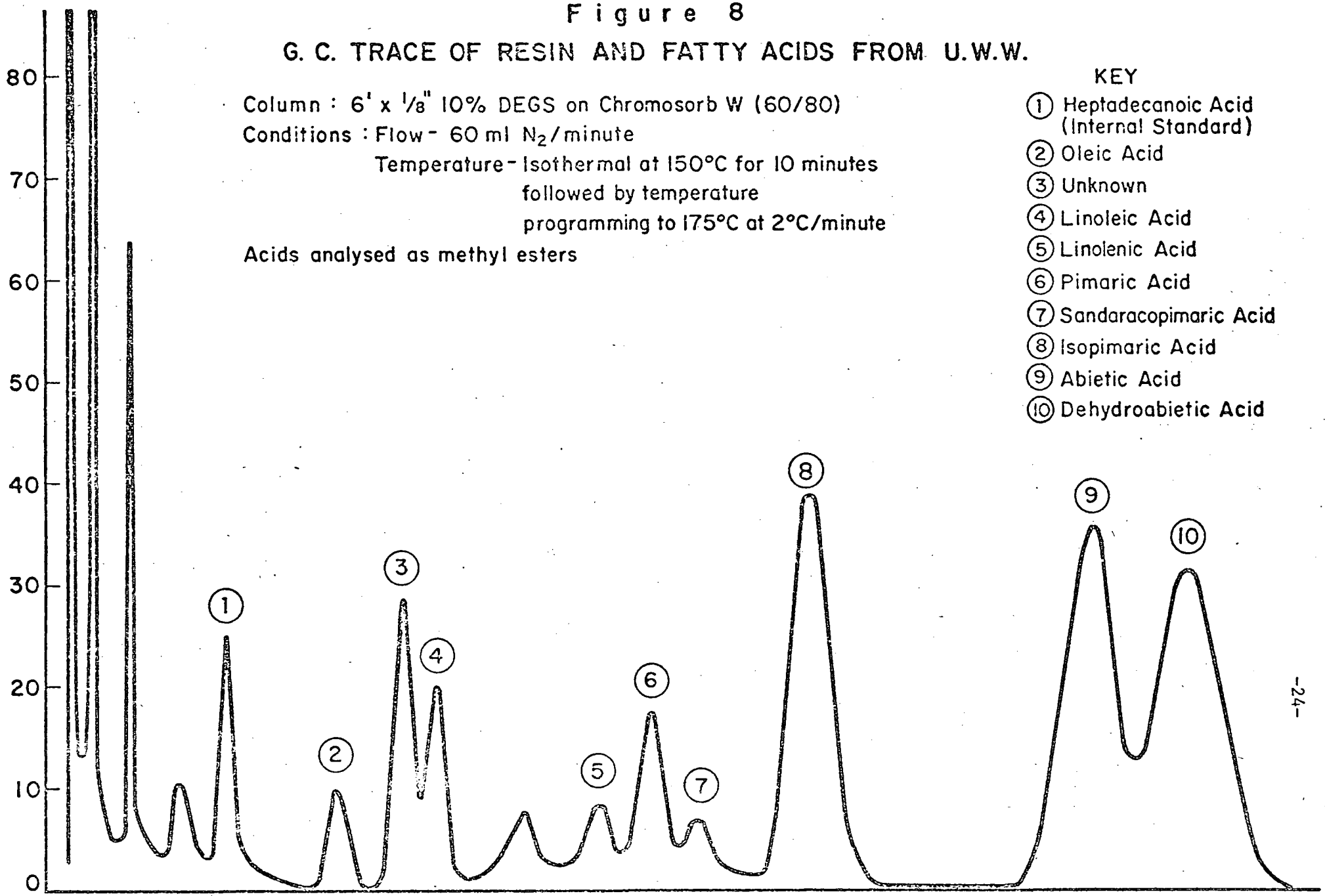
The useful lifetime of 10% DEGS columns was 6-8 weeks. Towards the end of this period, GC peaks due to pimarate and sandaracopimarate and peaks due to abietate and dehydroabietate tended to be imperfectly separated. Concentrations of these constituents in effluents were therefore sometimes given as the sum of pimarate plus sandaracopimarate and the sum of abietate plus dehydroabietate.

The optimum temperature range for separating fatty acid methyl esters was 120-150C, whereas resin acid methyl esters were resolved with reasonably sharp peaks at 170-180C. A compromise was made with the temperature programme that was finally adopted, viz. 10 min. at 150C followed by 2C/min rise to 175C. Individual fatty and resin acid methyl esters were adequately separated under these conditions so that accurate measurement of peak areas was possible. Using a nitrogen flowrate of 85 ml/min, a complete GC analysis occupied approximately 40 mins. A typical GC trace is shown in Figure 8. For some samples the instrument sensitivity was changed during the course of analysis in order to increase the areas of smaller peaks for more precise measurement. In such cases, response factors (see below) were calculated for standard resin or fatty acid solutions using the same instrument settings as for the sample.

Figure 8

G. C. TRACE OF RESIN AND FATTY ACIDS FROM U.W.W.

Column : 6' x 1/8" 10% DEGS on Chromosorb W (60/80)
Conditions : Flow - 60 ml N_2/minute
Temperature - Isothermal at 150°C for 10 minutes followed by temperature programming to 175°C at 2°C/minute
Acids analysed as methyl esters



(vii) Response Factors for Resin and Fatty Acid Methyl Esters

GC response factors were calculated frequently for resin and fatty acids by comparing peak areas due to standard solutions of the methyl esters with the peak area for a solution of the internal standard (heptadeca oic acid). Response factors differed for each acid and also varied for a particular acid from time to time. Values for resin acid methyl esters were in the range 0.85 to 1.55.

(viii) Spiking Samples

Some samples were analysed for resin and fatty acids in the usual way, then "spiked" with known amounts of pure resin acids and the analysis repeated. Recoveries of added acids were invariably greater than 95%. Many more samples will be spiked to ensure that good resin and fatty acid recoveries are obtained under a wide range of mill operating conditions.

(ix) Other Variables

In addition to the operating conditions discussed above, other variables require study. The capacity of XAD-2 resin for resin and fatty acids in the presence of other adsorbable materials has still to be determined. Thus, despite the success of "spiking" experiments described above, it is possible that certain constituents of pulp mill effluents will have a greater affinity for XAD-2 resin than the compounds of interest. Sites of adsorption may therefore be preferentially occupied or blocked by other materials, resulting in premature and unexpected leakage of resin and fatty acids. Experiments have also still to be conducted to confirm that optimum conditions are being used for fatty acid recovery.

Another variable which has not been examined is the extent to which XAD-2 resin can be regenerated and re-used. At present, resin is used once and then discarded. It would be helpful from the points of view of time and economics if a resin column could be used to process several samples.

(b) Application to Analysis of UWW Samples

UWW samples were obtained from eleven mills and analysed for resin and fatty acid content. Results of the analyses, together with information on sample toxicity and wood furnish are shown in Table 3. The geographical locations, pulp washing practices, wood furnish and operating conditions of the mills covered a broad spectrum. It is not possible, therefore, to attempt statistical correlations between resin and fatty acids and operating variables.

TABLE 3

RESIN AND FATTY ACID CONCENTRATIONS AND TOXICITY OF UWW SAMPLES

SAMPLE NO.	GEOGRAPHICAL LOCATION OF MILL	WOOD FURNISH	TOXICITY (MST-mins)		RESIN ACID CONCENTRATION (mg/litre)					UNSATURATED FATTY ACID CONCENTRATION (mg/litre)				
			100% CONC.	50% CONC.	PIMARIC + SANDARACO-PIMARIC	ISOPIMARIC	ABIETIC	DEHYDRO-ABIETIC	TOTAL	PALMI-TOLEIC	OLEIC	LINO-LEIC	LINO-LENIC	TOTAL
1	B.C. COASTAL	Douglas Fir Western Hemlock Western Red Cedar	21	46	0.3	3.7	1.5	1.0	6.5	ND	0.2	0.3	1.9	2.4
2	B.C. COASTAL	Douglas Fir Western Hemlock	37	53	0.2	1.7	← --- 3.6 --- →		5.5	ND	0.2	0.3	0.1	0.6
3	B.C. COASTAL	Douglas Fir Western Hemlock Balsam Fir Western Red Cedar	14	46	0.5	1.5	0.7	0.5	3.2	1.1	0.7	1.3	1.1	4.2
4	B.C. INTERIOR	59% Spruce 16% Pine, 11% Fir	7	26	2.3	12.3	6.7	8.5	29.8	0.6	0.9	2.3	1.2	5.0
5	B.C. INTERIOR	56% Pine 28% Spruce 14% Fir	-	-	1.3	1.0	8.4	4.9	15.6	0.7	0.3	0.1	ND	1.1
6	B.C. INTERIOR	45% Pine 50% Spruce 5% Fir	-	389	1.1	0.7	0.3	1.6	3.7	ND	0.3	0.1	0.7	1.1
7	B.C. INTERIOR	50% Lodgepole Pine 35% Spruce 15% Fir	290	>1000	0.2	0.6	← --- 1.5 --- →		2.3	0.1	0.1	ND	0.3	0.5
8	B.C. INTERIOR	20% Douglas Fir 43% Lodgepole Pine 28% Spruce 9% Alpine Fir	5	24	1.9	2.1	1.7	1.6	7.3	ND	0.7	4.2	3.0	7.9
9	ONTARIO	75-80% Jackpine 20-25% Black Spruce	15	27	3.5	9.3	17.9	15.1	45.8	ND	0.8	1.6	1.1	3.5
10	ONTARIO	Jackpine Dense Hardwood †	-	23	2.3	7.4	13.2	9.4	32.3	ND	3.2	10.6	1.9	15.7
11	ONTARIO	Jackpine Dense Hardwood †	-	22	3.1	5.4	19.9	9.2	37.6	ND	0.6	7.8	1.3	9.7
12	WASHINGTON	100% Ponderosa Pine	19	33	2.4	5.6	← --- 11.5 --- →		19.5	1.5	ND	ND	1.2	2.7
13	WASHINGTON	100% Ponderosa Pine	55	283	1.0	3.3	← --- 5.3 --- →		9.6	ND	0.4	0.6	0.5	1.5

ND = Not detected.
† Dense hardwood = birch, maple, elm, ash.

With the exception of 3 samples, all were extremely toxic, having MST values of less than 1 hour at 50% concentration. Generally, high toxicities were matched by high resin acid contents, though samples 3 and 8 had low resin acid concentrations and high toxicities. The same two samples were also unusual in having higher concentrations of C_{16} and C_{18} unsaturated fatty acids than resin acids. There are no hardwood species in the furnish at these two mills to account for the unusually high proportion of unsaturated fatty acids. Additional studies are planned in order to explain the anomalies.

Of the resin acids, abietate was commonly present in the highest concentration, though isopimarate or dehydroabietate was sometimes the major constituent. Linoleate was frequently found to be the predominant fatty acid, with linolenate next in overall abundance. Oleate and palmitoleate were usually minor constituents.

Spruce and pine species, especially jackpine, gave rise to UWW samples containing the highest total resin acid concentrations. Direct comparisons are not possible, however, since data on effluent volumes and production tonnages are not available. Attempts will be made in future work to obtain this information.

F. DISCUSSION

1. Fractionation and Identification of the Toxic Constituents of UWW

The toxic factors in UWW, produced during screening pulp from a cook of Douglas fir and western hemlock chips, were fully accounted for by maintaining material and toxicity balances throughout the chemical fractionation procedure. Log concentration - log MST graphs for separated and recombined chemical fractions were matched at all stages with bioassay data obtained for the original UWW (Figure 3). The chances of suffering undetected losses of toxicants were thus minimized. The excellent toxicity balance finally obtained using a synthetic solution of the toxic constituents in the correct concentrations is strong evidence that no toxicants of significance have been overlooked. Fractions labelled "non-toxic" in Figure 2 did not kill any fish after 96 hr exposure at concentrations 2-4 times those found in UWW. A considerable increase in the levels of constituents in the non-toxic fractions, relative to the toxic resin and fatty acid fractions, would be necessary therefore for other chemical compounds to become important contributors to UWW toxicity.

The toxicities of the resin and fatty acid fractions and those of the individual resin acid soaps were directly additive. No evidence was obtained for potentiation or antagonism between constituents (see Sprague, 1970).

There was a sharp decline in the contribution of the fatty acid fraction to UWW toxicity as concentration decreased. The four unsaturated fatty acid soidum salts responsible for the toxicity of this fraction gave a MST of 170 min. when tested at 6.2 mg/litre, the concentration at which they were found in UWW. However, at 90% of this level there were no fish mortalities. Thus, minor changes in the concentration of certain constituents can affect substantially the overall level of effluent toxicity. The wide variations in the toxicity of effluent from a mill, often observed over a short period of time, may be the result of small deviations in toxicant concentrations.

The compound, 4-(p-tolyl)-1-pentanol, was not found in the UWW, though it had previously been identified (Warren and Marvell 1961) as a toxic constituent of evaporator condensate from a west coast softwood kraft mill. Special efforts were made to detect the pentanol derivative for two reasons. Firstly, evaporator condensate studied by Warren and Marvell, and UWW examined in the present work have a common precursor, viz. black liquor. The constituents of the condensate from evaporation of black liquor would therefore be expected to be present in UWW from the brownstock washing, albeit in different concentrations. Secondly, wood species at mills in the two studies were similar. However, if 4-(p-tolyl)-1-pentanol did occur in the UWW of the current study, its concentration was too low to influence toxicity.

The practice, followed by many mills, of adding evaporator condensate to washwater on the brownstock washers was not used at the mill studied in detail in this project. The presence of condensate in UWW will not alter the range of constituents but will result in changes in their relative proportions. It is possible that under such circumstances, 4(p-tolyl)-1-pentanol might occur in sufficient concentration to influence toxicity. However, work done at B.C. Research under CPAR Project No. 10 (Bruynesteyn and Walden 1973) has shown that resin acid soaps are present in evaporator condensates. It is probable that resin acid soaps will be the major toxicants from the pulping of softwood species whether condensate is used in the washing step or not. Work is currently in progress in this connection.

The toxic materials that have been found in UWW are extractives from the wood chips and are not artifacts produced during pulping. Changes in the relative concentrations of individual resin acids may take place due to isomerisations during the cook, but it is likely that all the resin acids found in UWW occur in the original pulpwood. Differences in the concentrations of the wood extractives responsible for UWW toxicity may be expected as a result of changes in wood species (Assarsson and Åkerlund 1966). Thus, species having high proportions of resinous extractives, eg. jackpine, will give rise to pulping effluent (UWW) high in resin acid soaps, provided other process conditions do not vary greatly. Smaller effects could be caused by geographical location, the ratio of heartwood to sapwood in chips fed to the digester (Hancock and Swan 1965), or chip age (Rogers et al. 1971). Other minor influences on the extractives' content of the wood chips being cooked may be envisaged (eg. seasonal variations).

A more detailed investigation of the variables mentioned above may reveal the important factors affecting the concentration of resin acid soaps and, by inference, toxicity. Some indications of variations in toxicity and the composition of toxicants should be available as as result of examining UWW from various mills during the remainder of this project.

In view of the nature of the toxic constituents in UWW and the small concentrations in which they are found, there appears to be no chemical basis for assuming or searching for a relationship between effluent BOD_5 and toxicity.

There is no information in the literature on the toxicity of individual resin and fatty acid soaps towards salmonids. Some indication of the relative toxicities of certain resin and fatty acid sodium salts can be obtained on examination of Figure 4. Pimaric-type acids were more toxic than abietic-type acids. The toxicity of C_{18} fatty acid sodium salts increased with the level of unsaturation. Thus, stearate (C_{18} saturated) did not kill fish at 20 mg/litre whereas

oleate (C_{18} mono-unsaturated) was toxic at 12 mg/litre. Linoleate (C_{18} di-unsaturated) and linolenate (C_{18} tri-unsaturated) were successively more toxic. Palmitoleate (C_{16} mono-unsaturated) was the most toxic fatty acid soap tested, which may be an indication that toxicity also increases with decreasing chain length. However, soaps of the saturated fatty acids, laurate (C_{12}), myristate (C_{14}), and palmitate (C_{16}) were not toxic at concentrations up to 20 mg/litre. There was no evidence for the presence of unsaturated fatty acids below C_{16}.

Figure 4 also gives some idea of the incipient LC_{50} values (lethal threshold concentrations) for the various resin and fatty acid soaps. Threshold concentrations for resin acid sodium salts were approximately 1-3 mg/litre under the bioassay conditions used. None of the unsaturated fatty acid soaps were toxic below 9 mg/litre under the same test conditions.

2. Analysis of Resin and Unsaturated Fatty Acid Soaps in Effluents

(a) Method Development

Resin acid soaps have been implicated as toxicants in kraft effluents for many years. Various colourimetric procedures have been developed for their analysis. The most widely used technique in recent times has been that of Carpenter (1965). In this procedure, the effluent sample is acidified, extracted with petroleum ether, and the residue left on removal of solvent is redissolved in chloroform. Sulphuric acid (65%) and acetic anhydride are added to the chloroform solution and a transient magenta colour is produced in the acid layer. Resin acid soap concentrations are calculated with reference to a standard graph of absorbance at 525nm *vs* concentration obtained using abietic acid.

The shortcomings of the procedure are manifold. With respect to the manual operations involved, the initial extraction of resin acids from the effluent into petroleum ether is inefficient due to their poor solubility in non-polar solvents. In addition, inconvenient emulsification problems occur during the extraction and great care must be taken to avoid charring of the sample when removing solvent as described in the original method. Finally, the colour-developing agents are dangerous and unpleasant to handle, and the rate of colour formation and decay are temperature dependent and difficult to control.

Of more concern, the technique is neither specific nor selective for resin acids. Only conjugated diene acids give the desired colour reaction. Thus, many of the important resin acids, such as isopimaric, dehydroabietic and pimaric, do not register in the test (Stephens and Lawrence 1962). Furthermore the colour is given by many sterols, such β-sitosterol (Fieser and Fieser 1950), which is a common constituent

of pulping effluents, and also by some unsaturated fatty acids. A final drawback to the procedure is that abietic acid used to prepare the calibration curve is normally obtained from chemical suppliers as "Technical"grade crystals, of which 25-70% may be abietic acid and the remaining material a mixture of non-responding resin acids, such as dehydroabietic.

Thus, while the Carpenter technique has been the best available for resin acid analysis, there has been a need for a more sensitive and selective method. The identification of resin acid soaps as the major toxic factors in UWW has served to emphasize the importance of a reliable analysis for these compounds. Fortunately, the procedures which led to isolation of the toxic constituents also provided the basis of a viable method for their quantitative analysis.

The GC method for analysis of resin and fatty acids was developed with four major objectives. It was to be selective and free from interference, sensitive in order to detect sublethal levels, accurate insofar as good recoveries should be obtained, and rapid. All four objectives have been largely met by the procedure described in this report. Additional refinements are planned to ensure freedom from interference and good recoveries in all types of pulp and paper effluent, but the method as it now stands is adequate for many effluents. The technique has been used to measure the concentration of resin and long-chain fatty acids in several CPAR projects at B.C. Research. Numerous successful analyses have been carried out on unbleached whitewater, black liquor and evaporator condensates (Bruynesteyn and Walden, 1973), untreated and biologically treated bleached kraft whole mill effluent (Mueller, 1973), mechanical pulping effluent (Howard and Leach 1973) and woodroom effluent (Leach and Howard 1973). The time required for a complete analysis is approximately 3-4 hr., which is somewhat longer than the Carpenter method, but the information gained is far more accurate and provides greater detail, since individual resin and fatty acid constituents are measured. For many effluents, useful correlations have been obtained between the resin acid content and toxicity. Resin acid analyses have proven during the course of several CPAR projects to be a valid means of monitoring effluent toxicity.

Since the development of this procedure, the NCASI has issued details of a GC technique for resin acid analysis (NCASI, 1972). Sample preparation prior to analysis differs in the two methods. The NCASI procedure involves direct solvent extraction of the effluent, which may give rise to some of the problems discussed earlier in connection with the Carpenter method, as well the probability of introducing a more complex mixture of constituents into the GC.

(b) Results for UWW Samples

Total resin acid concentrations ranging from 2.3 to 45.8 mg/litre UWW were found in samples from eleven kraft mills. In the effluent used for large-scale isolation and identification of toxic factors, the resin acid concentration was 13.1 mg/litre. The C_{16} and C_{18} fatty acid concentrations in the mills surveyed were between 0.5 and 15.7 mg/litre. Relative concentrations of individual resin acids varied considerably. In this connection the major factor is thought to be wood species mix.

None of the mills studied were pulping chips high in hardwood content so no information is available at present on the concentrations of resin and fatty acid soaps or the toxicity of UWW derived from hardwood species. Fatty acids and their glycerides predominate over resin acids in hardwoods compared with the reverse situation in softwoods (Assarsson and Åkerlund, 1966). The proportion of fatty acid soaps in UWW from a mill pulping hardwood chips would therefore be expected to be higher than in UWW from a softwood mix. Investigations along these lines are in progress. In particular, efforts will be made to explain the resin to fatty acid ratios in Samples 3 and 8 (Table 3), which were anomalously low in view of the softwood furnish.

Total resin and fatty acid concentrations in kraft pulping effluents will also depend upon wood furnish and, in addition, will be governed by washing efficiences and the total volume of washwater.

The resin acid concentrations reported in Table 3 may be compared with the value of 2.6 mg/litre found by Mäenpää *et al.* (1968) for a similar effluent from a Finnish mill producing pine kraft pulp. Using GC analysis, Mäenpää *et al.* identified abietate and dehydroabietate as the main resin acid soap constituents. They also found small amounts of pimarate, palustrate and neoabietate, but did not detect isopimarate, a major constituent in many of the effluents tested in the current project. Whilst developing the large-scale fractionation scheme, one batch of UWW appeared to contain traces of neoabietate and either levopimarate or palustrate but they were not detected subsequently in other UWW samples. The thermodynamic instability of these compounds precludes their survival in substantial quantities at the pulping temperature of 170C.

In another study involving GC analysis of kraft effluents, Rogers (1973) detected dehydroabietate and isopimarate as major resin acid constituents, and lesser amounts of pimarate, sandaracopimarate and abietate in biologically treated bleached kraft whole mill effluent.

Over the normal pH range of UWW, most of the resin acids and all of the fatty acids will be present as their sodium salts. However, during the development of the large-scale fractionation procedure for UWW, trace quantities of methyl isopimarate and methyl abietate were detected in the effluent. Thus, the total resin acid figures quoted for UWW samples may include a small contribution from methyl esters. Various species of tree are known to contain minor amounts of resin acid methyl esters (Erdtman et al. 1968) and, since these compounds are saponified only with difficulty, they may survive the pulping process and emerge in UWW. The methyl esters were not toxic even when tested in amounts equal to the concentrations of resin acid soaps in UWW.

G. ACKNOWLEDGEMENTS

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