ENVIRONMENT CANADA

PROJECT REPORT 11-1

ISOLATION OF TOXIC CONSTITUENTS IN BLEACHED KRAFT EFFLUENTS

B.C. Research

Progress to January 15, 1971.

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 1970/71 on a project sponsored by the Water Pollution Abatement Research Program of the federal government in cooperation with the Canadian pulp and paper industry. First announced in August, 1970 by the Minister of Fisheries and Forestry and the Minister of Energy, Mines and Resources, this program provides for the funding of research contracts aimed at reducing pollution from Canadian pulp and paper operations.

The work summarized was funded by the Department of Fisheries and Forestry and the Department of Energy, Mines and Resources. Various elements of these Departments have recently been combined with the formation of the new Department of the Environment. The program Secretariat is provided by the Canadian Forestry Service, Department of the Environment.

The program is developed and guided by a joint government-industry committee known as the Coordinating Committee on Water Pollution Abatement Research (CCWPAR). The twelve members represent the Department of the Environment, the Department of Industry, Trade and Commerce, the Canadian Pulp and Paper Association, the pulp and paper industry in eastern Canada, the industry in western Canada and the Pulp and Paper Research Institute of 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. 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 of up to $500,000 were authorized to finance approved research work. From 1971/72 through 1975/76 up to $1,000,000 will be available each year provided the pulp and paper industry's annual expenditures for this type of work are increased by a like amount over the 1970 expenditures.

Project No. 1401

January 15, 1971

PROGRESS REPORT NO. 1

To: Department of Fisheries and Forestry
Room 4040, West Memorial Building
344 Wellington Street
Ottawa, Ontario

Attention Dr. H. Schwartz

Subject: CONTRACT NO. 7(CCWPAR)--ISOLATION OF TOXIC FRACTIONS IN UNBLEACHED WHITE WATER

A. OBJECT

To summarize progress on the above contract research program over the period, November 19, 1970 to January 15, 1971.

B. BACKGROUND

The research problem has been suitably described in our Project Proposal. The Proposal also included a description of information available through the literature and from unpublished studies undertaken in B. C. Research laboratories. The nature of the proposed investigation also was described. The appropriate portions of the Proposal have been excerpted and are attached as Appendix I.

The studies described in our Proposal were undertaken under sponsorship of a group of B. C. pulp and paper companies. After some interruption, these studies were reinitiated in the last half of 1970, still under industry sponsorship. Thus experimental studies were in progress when sponsorship was transferred from industry to the Federal Government on November 19, 1970. For the sake of clarity in this technical reporting, it is not feasible to differentiate absolutely between results derived before and after November 19. Similar difficulties will not arise with regard to future reports.

Previous studies on isolation of toxic fractions in kraft pulping effluents have been summarized by Van Horn (1961). Volatile toxicants include hydrogen sulfide, various organic sulfides and mercaptans. Resin soaps also have been recognized as major toxic

constituents of kraft pulping liquors (Hagman, 1936). Marvel and Wiman (1963) have estimated that 4-(p-tolyl)-1-pentanol is responsible for about 9% of the total toxicity. More recently, a number of constituents of toxic extractions of black liquor have been isolated and some of them identified by Banks (1969).

Even though Howard and Walden (1965) have shown that about 75% of the toxicity of mill effluent, as discharged, is attributable to abnormal pH, an appreciable proportion of the toxicity in unbleached pulping effluent remains unaccounted for.

In Appendix I, an effective procedure is described for the initial concentration of virtually all nonvolatile toxicity from kraft pulping effluents into a stable dry preparation, amenable to further fractionation. This procedure involves the petroleum ether extraction of the precipitate formed by acidiciation of effluent to pH 2. When the petroleum ether is removed by evaporation under nitrogen, the dry solid residue contains all the measurable acute toxicity. Repeated fractionation by column chromatography using a variety of solvent systems results in relatively pure preparations as judged by their IR and mass spectra and by their R_f values on thin layer chromatograms (TLC). Abietic acid has been identified positively as a major component, although other components were more toxic on a weight basis. Attempts to achieve a weight and toxicity balance proved inconclusive. Separation achieved on larger columns, necessary to provide sufficient material for a toxicity balance at each fractionation, was inadequate and final preparations were mixtures, rather than pure compounds.

The principal objective of the current study is to achieve a toxicity balance, as well as to isolate and identify toxic factors. Thus, since studies have been reinitiated, attempts have been made to secure in large amounts in a dry stable form, the material obtained by petroleum ether extraction of the acid precipitate formed from large volumes of effluent. At the same time, a toxicity balance has been sought on this preliminary fractionation. Subsequent purification by column chromatography is projected, using TLC to demonstrate chemical separation, solids values to evaluate weight fractionation and probit bioassays to demonstrate a toxicity balance.

C. EXPERIMENTAL

1. Large-Scale Separation of Petroleum Ether Extract of the Acid Precipitate

The large-scale separation, undertaken to secure material for continuing studies, involved 23,640 l of unbleached white water secured over a four-day normal operating period at a local coastal mill. During sampling, the mill was producing fully bleached kraft pulp.

a. Acid Precipitation

Effluent pH was adjusted to 2.0, which resulted in flocculation of the brown slimy precipitate. After settling overnight, the supernatant in individual vessels was decanted off and the sludge recovered. Total yield of sludge was 640 l.

Bioassay on initial aliquots of the original effluent and the decanted supernatant, using coho salmon fingerlings, as test fish, gave MST values at 100% concentration of 0.037 and <0.00066 min^{-1}, respectively. The latter value represents complete survival of test fish over a 1500-min exposure and is arbitrarily defined as being nontoxic. Over the four days that effluent was being processed, composite samples were prepared, representing the total volumes of original unbleached white water, the decanted supernatant and the recovered sludge. The decanted supernatant was then discarded.

Bioassays were undertaken at a number of dilutions, expressed as percent concentration in the original volume, of the composited samples of effluent, sludge and supernatant. The results were expressed as mean survival times in reciprocal minutes and the probit lines are plotted in Figure 1. Although the MST values, at 100% concentration, of the composited effluent and of the sludge agreed closely, these values were substantially lower than the value of 0.037 min^{-1} obtained for the origianl aliquot. These samples were not identical and most probably represent variation in mill effluent over the four-day sampling period, since acute toxicity is known to vary as much as 500% over a 24-hr period (Howard and Walden, 1971). However, in Figure 1 the slopes of the probit lines for the samples of composited effluent and of sludge are different. This infers that the separation has removed materials which were antagonistic to toxic factors, the toxicity of which has a limited susceptibility to dilution, or that some loss of these factors occurred during the separation. In addition, the composited supernatant showed some measurable toxicity at 100% concentration, albeit the toxicity was not great. The slope of this probit line is appreciably greater than those of the effluent or sludge, confirming the loss of factors of limited susceptibility to dilution, from the effluent to the supernatant. For example, at 30% dilution, the toxicities of the original effluent and of the supernatant are equivalent.

Thus, this larger-scale procedure has not achieved the separation obtained previously and obtained on the pilot-scale separation of the present study. The loss of toxic factors with limited susceptibility is also of concern. Although this loss probably did not occur previously,

inasmuch as no acute toxicity could be demonstrated, evaluation of probit lines for various fractions in this separation will be required as improved procedures are employed.

b. Freeze Dried Powder

In previous separations, the wet sludge obtained by acid precipitation and decantation of the supernatant was extracted serially with petroleum ether. Shaking of the sludge-water-petroleum ether system produced an extremely stable emulsion which could be broken only under high speed centrifugation. Because of the explosive hazards, centrifugation in refrigerated sealed containers was mandatory; an extremely laborious procedure with the volumes that had to be processed.

Attempts were made to eliminate the water remaining in the wet sludge. Finally, filtration was achieved on 13-in Buchner funnels, yielding 115 lb of wet cake. Nevertheless, filtration was difiecult because of the slimy nature of the sludge. Naturally occurring fiber in the effluent assisted filtration.

The wet cake was spread in layers 1/2-in deep on enamel trays and was deep frozen. The frozen plates were lyophilized in a Thermovac freeze dryer at a plate temperature not exceeding 40 C. The resulting weight of the dry material was 6.483 Kg.

The dry weight of the acid precipitate, exclusive of fibre, was determined by raising 1 liter of wet sludge to pH of 11.0 with 10% sodium hydroxide. The flocculated precipitate redissolved and the fibers remaining were removed by filtration and centrifugation. On re-acidification, the flocculated precipitate was centriguged out, deep frozen and lyophilized. The free-flowing black powder constituted 2.975 g/l of wet sludge, or 80.4 mg/l of the original effluent.

c. Petroleum Ether Extract

A portion of the freeze dried precipitate (221.2g) was extracted serially, four times, in a Soxhlet extractor for five hr at each extraction. Solids were recovered by evaporation of the petroleum ether in a Rotap evaporator, with removal of final traces of solvent under vacuum. Of the total solids recovered, 92% were removed in the initial extraction. Materials balance indicated that the petroleum ether extract represented 15.5 mg/l of the original effluent. Toxicity of the four fractions at a concentration of 18 mg/l was as follows:

Fraction	MST min^{-1}
I	0.0083
II	0.0118
III	0.0108
IV	0.0084
Extracted precipitate	<0.0002

Subsequently, 530 g freeze dried powder was extracted for 10 days with petroleum ether to yield 30 g of a semisolid yellow wax.

d. Discussion

These studies are being pursued with the overall object of isolating and identifying the toxic constituents of kraft effluents. At the same time, all toxic constituents are to be accounted for, and their relative role in the toxicity of the eflfuent determined. Both purposes are essential. It is anticipated that a complete accounting of all toxic principles can be achieved by assessing the effects of dilution, and the ratio of toxicities of individual isolates, as measured by acute and sublethal tests.

Previous preliminary separations, albeit on a smaller scale, resulted in complete recovery of the nonvolatile toxic principles in kraft pulping effluent. Indeed, the small-scale separation made in the current study to ensure the validity of the technique on the effluent used, yielded an identical result. Nonetheless, the data for this larger-scale separation clearly demonstrate considerable loss of biological activity. Logically, the losses are associated with longer time periods involved in larger-scale separations, possibly more extreme conditions, or both.

The principal processing delays associated with this larger-scale separation are as follows:

i. Collection and precipitation of effluent (four days)
ii. Dewatering of sludge (up to two weeks)
iii. Extraction of freeze dried precipitate (10 days)
iv. Elimination of petroleum ether extractant.

The freeze dried acid precipitate and the semisolid residue recovered from the petroleum ether extraction of the freeze dried acid precipitate should be amenable to storage with a minimum loss of activity. Storage at reduced temperature and under nitrogen may prove helpful.

To keep sampling and precipitation time to less than eight hr, for two men in the field, it has been decided to process effluent lots of only 5000 l. This is expected to produce approximately 1 Kg of freeze dried powder, representing about 400 g of the dried acid precipitate. Examination of various filtration techniques demonstrated that the wet sludge accruing from acid precipitation is handled best by an initial gravity filtration, which allows the fibres in the sludge to settle on the filter medium and form a mat, followed by vacuum filtration. Available small-scale commercial equipment did not fulfill these requirements, so two large Buchner filters have been constructed from 45-gal drums. Filtration of sludge from 5000 l of effluent is expected to take eight to 16 hr. Thus, within 36 hr of sampling, the filtered acid precipitate is expected to be in the frozen state, prior to drying. Fractionations, in the field and based on these procedures, are now in progress.

The freeze dried acid precipitate will be suspended in petroleum ether and serially extracted in the cold by intimate mixing, over a total time period not exceeding 3 hr. Large-scale equipment for flash evaporation of the petroleum ether is available. Final removal of the last traces of ether will be by Rotap evaporation at room temperature. These latter techniques will be piloted by small-scale trials and the separation achieved at each step of the entire fractionation will be monitored by bioassays at three concentrations.

2. Preliminary Chromatographic Separations

Studies, described in Appendix I, achieved excellent small-scale separation of the petroleum ether extract of the acid precipitate (PEAP), using silica gel columns and a variety of solvent systems. With larger-scale trials, separations were less clear cut. Therefore, using the semisolid yellow wax, which represented the PEAP obtained from the large-scale separation described herein, a DEAE Sephadex column was evaluated. Using the procedure described by Zinkel and Rowe (1964) a separation can be made, based on functional groups, i.e., hydrocarbons, long chain fatty alcohols, fatty acids, phenols and resin acids. A 30-g portion of the semi-solid yellow wax, dissolved in 100 ml of 89:10:1 diethyl ether: methanol:water, was put on a column loaded with 150 g of pre-treated DEAE Sephadex. Development was at 10 - 15 C, using the same solvent system. A total of 37 fracions were collected and the contents of each assessed by thin layer chromatography. Individual TLC's were developed with a solvent mixture containing equal volumes of petroleum ether and diethyl ether. Individual spots were identified with a nitric-sulfuric acid spray, followed by heating, or by antimony pentachloride in carbon tetrachloride. Individual fractions were grouped into six lots, based on the TLC's.

The first four fractions, which contained 43% of the material charged to the column, consisted of long chain hydrocarbons, fatty alcohols and probably β-sitosterol. Fractions 5 and 6 contained principally long chain substituted hydrocarbons and β-sitosterol; whereas relatively minute amounts of solids were contained in Fractions 7 to 22 inclusive. Fractions 23 to 26 inclusive, which contained 8% of the starting material, consisted chiefly of phenolics, fatty acids and resin acids. Fractions 27 to 37 inclusive contained fatty acids and resin acids and represented 40% of the starting material. Preliminary bioassay tests showed that toxicity resided exclusively in Fractions 23 to 37 and that Fractions 1 to 22 were nontoxic.

Thus this particular procedure is highly useful for separation of neutral from acidic molecules. Separation was expected between fatty and resin acids, which was not achieved. Alternate pretreatment of the DEAE Sephadex is expected to improve this separation, to be followed by separations worked out previously on silica gel columns. A materials balance of the separations achieved during the preparation of PEAP and its fractionation on a DEAE Sephadex column is presented in Table 1.

D. FUTURE RESEARCH

Work is in progress to produce suitable quantities of PEAP in a dry stable form with minimal loss of biological activity during preparation. The materials balance achieved to date will be supplemented by a toxicity balance, using probit bioassays to demonstrate total toxicity recovery.

Subsequently PEAP will be fractionated on a DEAE Sephadex column, prior to fractionation on silica gel columns, as per procedures developed previously. Efficacy of individual separations will be checked by thin layer chromatography and both a material balance and a toxicity balance will be sought. Possible antagonism and synergism affecting the toxicity balance at individual separations will be examined by comparing probit lines, before separations are continued. Isolates will be identified by infra red, NMR and mass spectroscopy, supplemented by elemental analysis, as necessary. Behaviour of isolates to various toxicity bioassays, at various concentrations, is expected to demonstrate whether the isolation has failed to account for any sublethal toxicity.

Succeeding studies are expected to include elaboration of chemical methods of measuring the toxic isolates, based on identification of their chemical structure. Additional work on acid and caustic bleach effluents is contemplated, although no detailed planning has been undertaken.

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F. ACKNOWLEDGEMENT

We should like to acknowledge the assitance of Dr. H. Rogers, Forest Products Laboratory, Vancouver with relation to column chromatographic separations.

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CCW:c

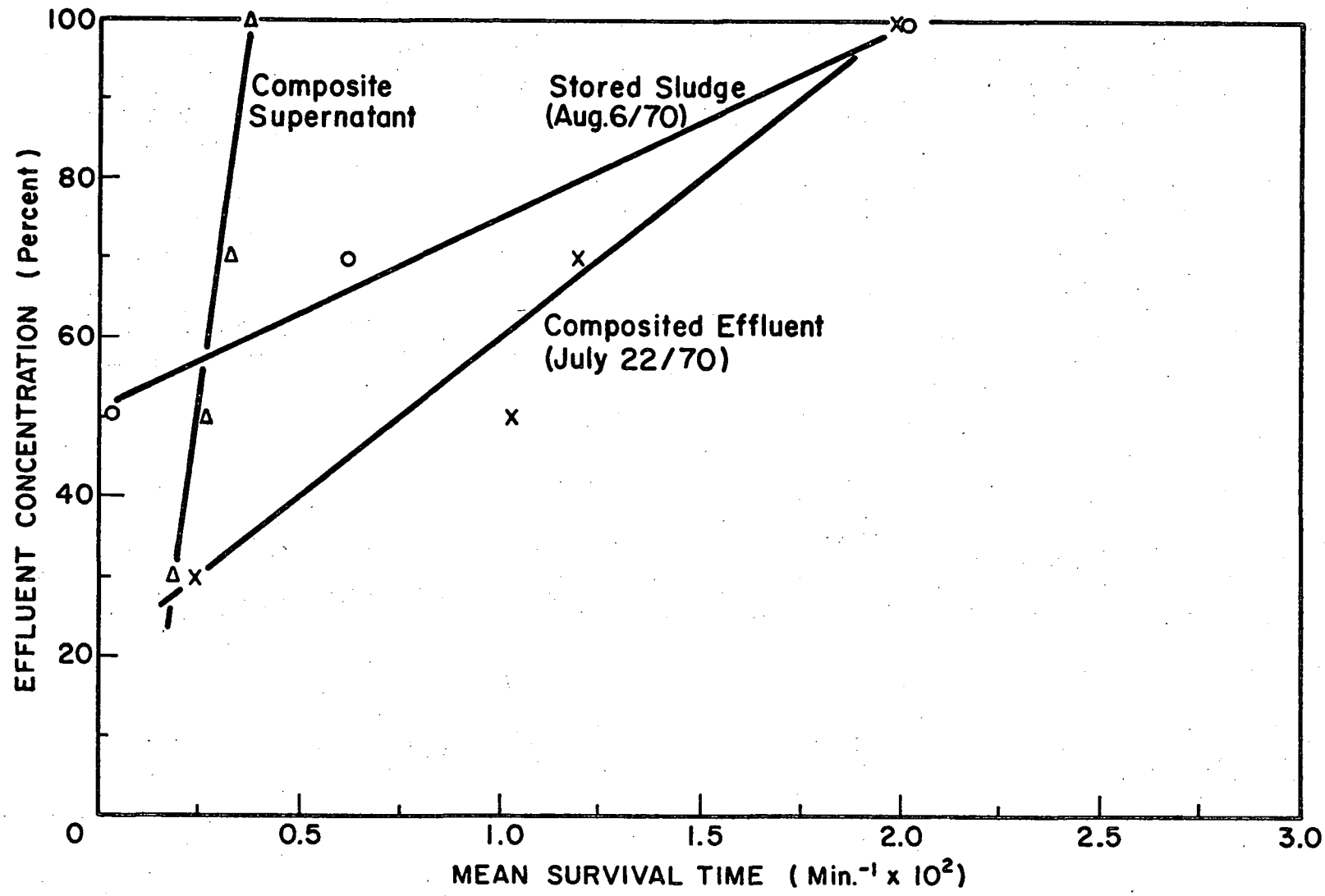
Table 1

CONSTITUENTS OF UNBLEACHED WHITE WATER
COLLECTED DURING A FOUR-DAY PERIOD OF NORMAL KRAFT PULP MILL OPERATION

Constituent	mg/l
Fiber	204
Fiber-free acid precipitate	80.4
Petroleum ether extract of acid precipitate	15.5
Hydrocarbons, neutrals	6.8
Phenols	0.6
Resin acids and fatty acids	6.5

Figure 1

THE MEAN SURVIVAL TIME AT VARIOUS CONCENTRATIONS OF COMPOSITED EFFLUENT AND OF SLUDGE AND SUPERNATANT FORMED BY ACID TREATMENT OF EFFLUENT TO pH 2.0



APPENDIX I

Project Proposal

1. Title of the Project

Isolation of toxic constituents of kraft pulp mill effluents.

2. Statement of the Problem

The toxicity of bleached kraft pulp mill effluents is due to various molecular entities; some inorganic such as bivalent sulfur, some organic such as the resin acids. The bulk of the toxicity, which is due to nondistillable organics, has not been identified in terms of molecular constitution.

The advantages of obtaining such information include better definition of the safe lethal and sublethal limits of concentration to fish and other aquatic fauna. The complex interaction of mixtures of toxicants, which may involve synergistic or protective effects, can be evaluated more readily where the nature of the toxicants has been established. With elaboration of the molecular identities, chemical techniques may be evolved for their quantitative measurement, in process and effluent streams. Such techniques hold considerably more promise for control over release of toxicants, than do corresponding biological procedures.

3. Currently Available Technology

a. Pulping Effluent

Sulfides, mercaptans, dimethyl disulfide and sulfur compounds in other oxidation states have been implicated frequently as toxic agents contained in kraft pulping waste liquors. Although no doubt exists as to the presence of these materials in pulping process streams, or their toxicity, our experience indicates that they are of little practical significance, due to rapid loss by direct air stripping or oxidation, in the immediate vicinity of the outfall or during external-to-plant waste treatment. Other compounds have been listed as important toxicants, but again their relevance in practical mill operating situations is doubtful. For example, sodium sulfate is cited as toxic at the 10,000-ppm level, a situation which will never occur in the natural environment. Economic operating practice now dictates that sewer flows are limited essentially to main process streams and that spillage of black liquor and dregs, and the toxicity associated with these spills, can be limited to a minimum by in-plant control.

Despite variations in operating procedures and design within individual mills, the main sewer from the pulping section of a kraft mill intrinsically represents the excess of weak wash water from the brown stock screening and deckering area as the unbleached pulp is transported from the digesters to the driers or to the bleach plant. It is probably valid to consider this effluent as an extremely dilute black liquor, escaping recovery by virtue of dilution with pulp transport water within the kraft pulping system.

In an extensive study of the toxic characteristics of unbleached kraft pulping sewers, Warren and Marvell[1] demonstrated that the toxicity was due mainly to nondistillable components. Among a number of preliminary purification procedures, these workers included the adjustment of alkaline wash water samples to pH 4 with subsequent petroleum ether extraction. This procedure, together with others examined, was described as giving inconclusive results.

We have demonstrated that all measurable acute toxicity in kraft pulping effluents can be recovered by acid precipitation at a pH 1.5 - 2.0 and subsequent petroleum ether extraction of the precipitate. Using column chromatography and various solvent systems, the ether extract of the acid precipitate has been split into 10 - 11 individual compounds, whose chemical behaviour suggests that they are individual chemical entities. Chromatographic procedures were established by preliminary thin-layer chromatography studies. Subsequent isolations from several thousand gallons of kraft pulping effluent have produced quantities of the two major toxic compounds, in amounts of the order of several hundred milligrams. As purification procedures were developed, toxicity and solids contents were determined, so that at the present time, an approximate material and toxicity balance can be described.

For kraft pulp wash water, obtained from a coastal mill pulping mixed softwood chips, the toxicity can be assigned to two major components. The minor component is a white translucent crystalline material, slowly soluble in petroleum ether. Although the infrared spectra of the isolate and abietic acid are identical in many respects and similar in others, the R_f values, when run in acid and basic mixed solvent systems, are not. The isolated material represents approximately 5% by weight of the petroleum ether soluble material, extracted from the original acid precipitate. At a concentration of 10 ppm, the time to death for guppies (Lebistes reticulatus) is 240 - 600 minutes, comparable to technical grade abietic acid.

The major toxic component has not been identified at the present time. Quantitative microorganic analysis and mass spectrometry indicate an aliphatic-type compound, possessing an active carboxyl group compound with a molecular weight of 302 and an empirical formula of $C_{20}H_{30}O_2$. Despite the formula, the material does not display any of the typical reactions of resin acids and present indications are that it is a branch chain fatty acid, with some unsaturated bonds. The infrared absorption spectra for this compound are different from the spectra of the first isolate. The material represents approximately 5% by weight of the petroleum ether soluble material, obtained from the original acid precipitate. At a concentration of 10 ppm, the time to death of test guppies is 120 - 170 minutes.

The preliminary toxicity balance suggests about 85% of the toxicity has been accounted for. Certain anomalies, suggesting synergism, have not been resolved. Attempts to extend column chromatographic

separations to a scale providing adequate material for a complete toxicity balance has not yielded the same degree of separation as previously, as evidenced by mass spectrographic data.

b. Bleach Plant Acid Effluent

Conventional bleaching processes result in an approximate 5% weight loss in processed pulp. The acid bleach effluent is the dominant process sewer in a bleached kraft pulp mill, as expressed in percentages of total flow and BOD_5, for the whole mill.

Only limited studies concerning the toxic components of kraft bleaching effluents have been made. Servisi et al[2] in examining the effects of chlorinated catechols on salmon, failed to demonstrate the presence of these materials in bleach effluents. Preliminary studies in our laboratory do not demonstrate the presence of chlorinated catechols.

Das et al[3] have presented strong evidence of the presence of tetrachloro-o-benzoquinone as a toxic constituent in first stage chlorination effluent. Despite the observation of Betts and Wilson[4] regarding the prime toxicity of acid bleach effluent in the particular mill under study, Howard and Walden[5] in a study embracing seven mills showed the acid bleach effluent to be the least toxic major effluent stream. Scrutiny of the bioassay data of Das et al[3] suggests considerable toxicity in the acid bleach effluent may not be accounted for.

A preliminary separation procedure has been developed in our laboratory, based on direct petroleum ether extractions of the acid bleach effluent and isolation of toxic fractions, using column chromatography, combined with various solvent systems.

Although the initial petroleum ether extraction removes all of the measurable acute toxicity from the effluent; this technique is not considered to be practical for processing the volumes of effluent necessary for complete isolation and identification studies. At the present time, the purity of individual fractions has not been established and no identification of toxic materials has been attempted.

c. Caustic Extraction Effluents

The second major process stream in kraft bleaching operations contains the chlorinated lignin derivatives, which have been solubilized by caustic extraction. We have considerable data to show that the toxicity of this stream is highly variable between individual mills. However, virtually no research activity concerning the toxic components of this sewer has been reported in the literature. None has yet been carried out in our laboratories.

4. Prime Objectives

This study will complete isolation and identification of toxic fractions in brown white water, concurrent with a toxicity and materials balance on

the isolation procedures to demonstrate that all toxic fractions have been accounted for. Similar studies will be initiated on acid and caustic bleachery effluents.

5. Nature of the Investigation

a. Present studies on toxic constituents in unbleached pulping effluents will be completed. This will involve identification, of the two isolates responsible for about 85% of the measurable acute toxicity. Within practical limits, molecular structure will be elucidated by mass and nuclear magnetic resonance spectroscopy, together with related chemical tests. Without attempting to identify other consituents of negligible toxicity, a quantitative material and toxicity balance, involving all isolates and purification fractions, will be sought.

b. As practical, assessed on the basis of the molecular identity of isolated toxicants, chemical techniques will be sought for their quantitative estimation in mill process streams and effluents.

c. Alternative procedures for extraction of toxic principles from acid bleach effluents will be sought, as a prerequisite to subsequent studies intended to isolate the pure compounds involved, and to establish their chemical identity. If techniques, capable of processing the volumes of raw effluent necessary in the preliminary purification stages are not available, then a material and toxicity balance will be sought on the existing purification scheme.

d. Studies, similar to those outlined in steps 1 and 2, will be commenced with caustic extraction effluents. The application of effort involved will depend upon initial progress. However, it is anticipated that some preliminary purification techniques can be evolved, which can be documented on the basis of associated toxicity and solids content.

e. Utilizing the acute bioassay, the respiration bioassay, together with other assays involving sublethal effects which may become available at that time, a representative number of toxic fractions, obtained as in steps 1 to 3 above inclusive, from pulping, acid bleach and caustic extraction effluents, will be examined from a number of mills. The ratio of toxicity to solids and acute toxicity to sublethal response will be examined to assess any residual sublethal activity, not accounted for by the concentration procedures. This basis will be used to define toxicity weight ratios in meaningful absolute terms.

6. Timetable

This investigation will take five years. It is anticipated that annual submissions will be made for support and that continuing support will be based on progress towards achieving the prime objectives of the investigation.

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