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Project Summary

Development of a Chemical Toxicity Assay for Pulp Mill Effluents

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A chemical analysis procedure was developed to rapidly measure compounds responsible for the toxicity of pulp mill effluents to fish. These results were used to estimate effluent toxicity as measured by standard laboratory bioassays, and to determine loadings of various toxic compounds from all the pulp mills on two river systems.

The analytical procedure involved adsorption of toxic compounds from effluent onto Rohm and Haas Amberlite XAD-2 polymeric resin, elution of adsorbed constituents from the resin with ether and methanol, methylation of the extract, and quantitative analysis by glass capillary column gas chromatography. Analysis time was less than one and one-half hours.

Results for 113 samples of raw and biologically-treated bleached and unbleached kraft, sulphite and groundwood effluents were converted *via* toxic units into estimates of acute lethal toxicity. Agreement between 96-h median lethal concentrations calculated by this method and values from bioassays of the effluents using rainbow trout was within ± 30% for 73% of the samples examined. Calculated 96-h LC50's and those measured by bioassay differed by more than 30% for the other 27% of samples examined. At present, therefore, toxicity determined from chemical analysis can be a valuable supplement to, but not a substitute for, direct measurement by bioassy. Samples were collected and analysed for five consecutive days from all pulp mills on the Willamette River, Oregon, and the Androscoggin River flowing through New Hampshire and Maine, to determine loadings of known toxic compounds into and out of the biobasins. Biotreatment at most of the mills was very effective in removing toxicants Estimates based on theoretical dilution capacity of the receiving waters showed that secondary treatment was highly beneficial in protecting the rivers against the toxic effects of pulp mill effluents. Minimum dilution of pulp mill effluents discharged to the Willamette River during the study period (October, 1979) was 93-fold, assuming rapid and complete mixing. In the Androscoggin River, the minimum effluent dilution during the study period (February, 1980) was 17-fold.

This Project Summary was developed by EPA's Industrial Environmental Research Laboratory, Cincinnati, to announce key findings of the research project that is fully documented in a separated report of the same title (see Project Report ordering information at back).

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Introduction

In recent years, more than 30 organic compounds have been identified as contributing to the toxicity of pulp mill process effluents to fish. Chemical analysis procedures using gas chromatography (GC) have been developed for toxicants in the various waste streams, and toxicity of the effluents to fish has been estimated by summing toxic unit equivalents of the measured toxicant concentrations. For many of the streams studied, good correlation has been obtained between the toxicity calculated from results of GC analyses and values obtained from acute lethal bioassays using rainbow trout.

As an extension of the previous work, the primary objectives of this study were to:

A. Develop a chemical analysis procedure for rapid measurement of the concentrations of toxic materials in bleached and unbleached kraft whole mill, groundwood, and sulfite mill effluents.
B. Relate the chemical assay results to effluent toxicity measured in bioassays using rainbow trout.
C. Measure toxicant loadings from kraft whole mill effluents.
D. Measure toxicant loadings from groundwood and sulfite mills.
E. Make a preliminary assessment of the environmental significance of mill biotreatment systems for reducing toxic loadings in effluents.
F. Measure toxicant loadings from all mills on selected river systems.

The work was carried out in two phases; (I) development and evaluation of an analytical procedure for predicting effluent toxicity (objectives A, B); (II) use of the procedure for estimating toxicant loadings (objectives C-F).

Conclusions

Phase I

The sample (20-50 ml of untreated effluent; 100 ml of biotreated effluent) adjusted to pH 9 with 10% NaOH solution, and to a specific conductance greater than 2 mmho/cm using saturated NaCl solution, was passed from a 250-ml separatory funnel at 15 ml/min through a glass column (1.6 x 22 cm) containing Amberlite XAD-2 resin (12 ml), previously rinsed with deionized water (3 x 15 ml). The funnel was rinsed once with a small amount of NaCl solution at pH 9, and residual effluent was expressed from the column using a stream of nitrogen. Adsorbed constituents were extracted by allowing 9:1 ether/methanol (15 ml) to stand in contact with the resin for 10 min before eluting into a 100-ml glass centrifuge tube at a flow rate of 15 ml/min. Additional ether/methanol (2 x 10 ml) was then passed through the resin to complete the extraction of adsorbed toxicants. Effluent trapped by the resin and eluted during passage of solvent was separated from the solvent layer by immersing the centrifuge tube in an acetone/dry-ice bath for 2 min. The organic layer was decanted from the frozen aqueous layer into a 100-ml round-bottom flask. The ice was thawed, washed with diethyl ether (2 x 5 ml), frozen again, and the organic extracts were combined.

Heptadecanoic acid (1 ml of a solution containing 0.05 mg/ml in methanol) was added to the solvent extract as an internal standard. The solvent was evaporated almost to dryness using a rotary evaporator, transferred using ether (6 ml) to a 15-ml graduated centrifuge tube, and concentrated to 2 ml by gentle warming on a water bath. Methanol (0.2 ml) was added and the solution was methylated with diazomethane for 10 min. The solution was evaporated to 0.1 ml on a warm-water bath, and aliquots (2-5 μl) were analysed by capillary column GC using a Hewlett-Packard Model 5830 FID instrument. Column specifications were: 16 m glass (0.25 mm i.d.) WCOT OV-101; initial temperature, 190°C, programmed at 1°C/min (20 min), 2°C/min (10 min), then 3°C/min to 250°C. Toxic compounds of interest were identified routinely by matching retention times with those of standards using a Hewlett-Packard Model 18850A integrator terminal with settings of attenuation 16, slope sensitivity 0.1, area reject 100, and retention time window 2%. Identities of the constituents were authenticated in some samples by combination gas chromatography-mass spectrometry and in others by confirming that retention times coincided in samples "fortified" with added amounts of the pure compounds. Chlorinated guaiacol concentrations were checked by electron-capture GC (Hewlett-Packard Model 5710 instrument containing a glass column, 0.32 cm x 1.8 m, packed with 8% OV-1 on Chrom W (HP), 80-100 mesh; temperature, 175°C; carrier gas 95% argon, 5% methane; flow rate 20 ml/min) using aldrin as an internal standard. Samples from each mill were analysed before methylation to detect neutral compounds. Analysis time, excluding this last step, was 1 h 25 min.

Recovery efficiency was measured using portions of the effluents to which known amounts of the toxic constituents had been added. Aliquots (4 or 8 ml) of stock solutions containing each toxicant in methanol (0.25 mg/ml) were mixed and diluted to 20 ml with methanol to give a solution containing 0.05 or 0.10 mg/ml of each compound. An aliquot (1 ml) of this solution was added to the effluent sample 20-100 ml), which was then processed through XAD-2 resin.

Recovery efficiencies were in the range of 80-103% for toxicants added to effluent samples, reflecting a level of accuracy compatible with other complex environmental analyses of organic constituents.

Analytical conditions were varied during development of the procedure to optimize accuracy and minimize analysis time for aqueous standard solutions of toxic constituents and for effluent samples. Effects of the following variables on analytical accuracy and precision were studied:

1. Ionic strength and pH of toxicant solution or effluent.
2. Use of ether or ether/methanol for elution of adsorbed materials from XAD-2 resin.
3. Solvent evaporation technique.
4. Drying technique.
5. Internal standard for GC analysis.
6. GC conditions.
7. Variation of GC retention time and response factor with time and concentration.

Analytical procedures were evaluated for mixtures of the following compounds, which have been identified as important toxic constituents of various pulp mill process streams.

abietic acid
dehydroabietic acid
isopimaric acid
pimaric acid
sandaracopimaric acid
oleic acid
linoleic acid
linolenic acid
juvabione
juvabiol
dihydrojuvabione
pimarol
isopimarol
trichloroguaiacol
tetrachloroguaiacol
monochlorodehydroabietic acid
dechlorodehydroabietic acid
epoxystearic acid
dichlorostearic acid

Primary- and secondary-treated bleached and unbleached softwood kraft and sulphite whole mill effluents from 26 mills (Table 1) were then analysed

Table 1. *Mills from which Samples were Obtained for Bioassay and Analysis*

Code	Mill Location	Type	Effluent Sample
A	British Columbia Coastal	Unbleached softwood kraft	Primary
B	Oregon	Bleached softwood kraft	Primary
C	British Columbia Interior	Bleached softwood kraft	Primary
D	British Columbia Interior	Bleached softwood kraft	Primary, secondary
E	British Columbia Interior	Bleached softwood kraft	Primary, secondary
F	British Columbia Interior	Bleached and unbleached softwood kraft	Primary, secondary
G	Mid-West USA	Bleached hardwood kraft	Secondary
H	East Coast	Unbleached low-yield sodium-base sulphite (softwood)	Primary
I	Ontario	Unbleached low-yield sodium-base sulphite (softwood)	Primary
J	Ontario	Bleached calcium/magnesium-base sulphite (softwood)	Primary
K	Maritimes Canada	Bleached ammonium-base sulphite (softwood)	Primary, secondary
L	N. Carolina	Bleached softwood and hardwood kraft	Primary, secondary
M	British Columbia Interior	Refiner groundwood	Primary, secondary
N	Washington	Bleached and unbleached softwood kraft; NSSC	Primary, secondary
O	Mid-West USA	Bleached hardwood kraft; hardwood NSSC	Primary, secondary
P	Washington	Bleached calcium-base sulphite (softwood)	Primary, secondary
Q	Oregon	Bleached ammonium-base sulphite (softwood)	Primary, secondary
R	Washington	Bleached ammonium-base sulphite (softwood)	Primary
S	Western Canada	Unbleached softwood kraft	Primary
T	Washington	Refiner groundwood (softwood)	Primary, secondary
U	Oregon	Unbleached softwood kraft; NSSC	
V	Oregon	Unbleached magnesium-base sulphite; refiner groundwood (softwood)	Primary, secondary
W	Oregon	Unbleached softwood kraft	Primary, secondary
X	Washington	Refiner groundwood	Primary, secondary
Y	Oregon	Bleached softwood and hardwood kraft	Primary, secondary
Z	Washington	Bleached softwood kraft and magnesium-base sulphite	Primary, secondary

Toxicity of the effluents to rainbow trout were measured by bioassay, and the results were similar to values calculated from concentrations of the individual toxicants, using the toxic unit concept.

Agreement between 96-h LC50's estimated from analysis results and the values from bioassays was within 30% for 73% of 113 effluent samples examined (Figure 1). For approximately 10% of the samples, analysis results predicted a high toxicity value whereas bioassay indicated low toxicity, or vice versa. Resin acids were the predominant toxic constituents in primary-treated effluent samples from mills operating on softwood species. Levels of these compounds were generally, though not invariably, a good indicator of the effluent toxicity. None of the secondary-treated effluent samples examined from mills in the United States were toxic to rainbow trout.

Phase II

The analysis procedure was then used to measure the concentrations of potentially toxic compounds in the influents to and effluents from biotreatment ponds at all mills on the Willamette River, Oregon, and the Androscoggin River, New Hampshire and Maine (Table 2). The results were used to assess, in a preliminary way, the environmental significance of mill biotreatment systems on the two rivers. Retention times in the various biobasins were from 6 hours to 14 days.

All except one mill on the Willamette River and one on the Androscoggin River provided excellent removal of the toxic compounds analysed. Calculated total daily loadings discharged from all mills to the Willamette River were 2 kg chlorinated guaiacols, 52 kg C_{18} unsaturated fatty acids, and 71 kg resin acids. Approximately 20% of the fatty acids and 67% of the resin acids originated from one mill. Secondary treatment systems at the remaining mills removed 60-90% of unsaturated fatty acids and 85-99+% of resin acids, based on loadings in influents and effluents.

Calculated total daily loadings discharged from all mills to the Androscoggin River were 55 kg C_{18} unsaturated fatty acids, 115 mg resin acids, 21 kg tri- and tetra-chlorinated guaiacols, and 2.7 kg chlorinated dehydroabietic acids. One mill accounted for 56% of the resin acid loading to the river. Secondary treatment at mills on the Androscoggin River removed 91-96% of the unsaturated fatty acids, 92-97% of resin acids (except at one mill where resin acid removal was 80%), and amounts of the chlorinated guaiacols and chlorinated resin acids ranging from 50-90%.

Table 2. *Mills Sampled for Toxicant Loadings Studies*

Willamette River, Oregon
- *Unbleached kraft*
- *Unbleached magnesium-base sulfite, refiner groundwood, and de-inking (2 mills)*
- *Stone groundwood*
- *Unbleached kraft/NSSC*
- *Bleached kraft*
- *Defibrated wood pulp*
- *Bleached ammonium-base sulfite*
- *----all softwood mills*

Androscoggin River, New Hampshire, Maine
- *Bleached softwood and hardwood kraft*
- *Bleached softwood and hardwood kraft, groundwood*
- *Refiner groundwood*
- *Bleached softwood and hardwood kraft, NSSC*

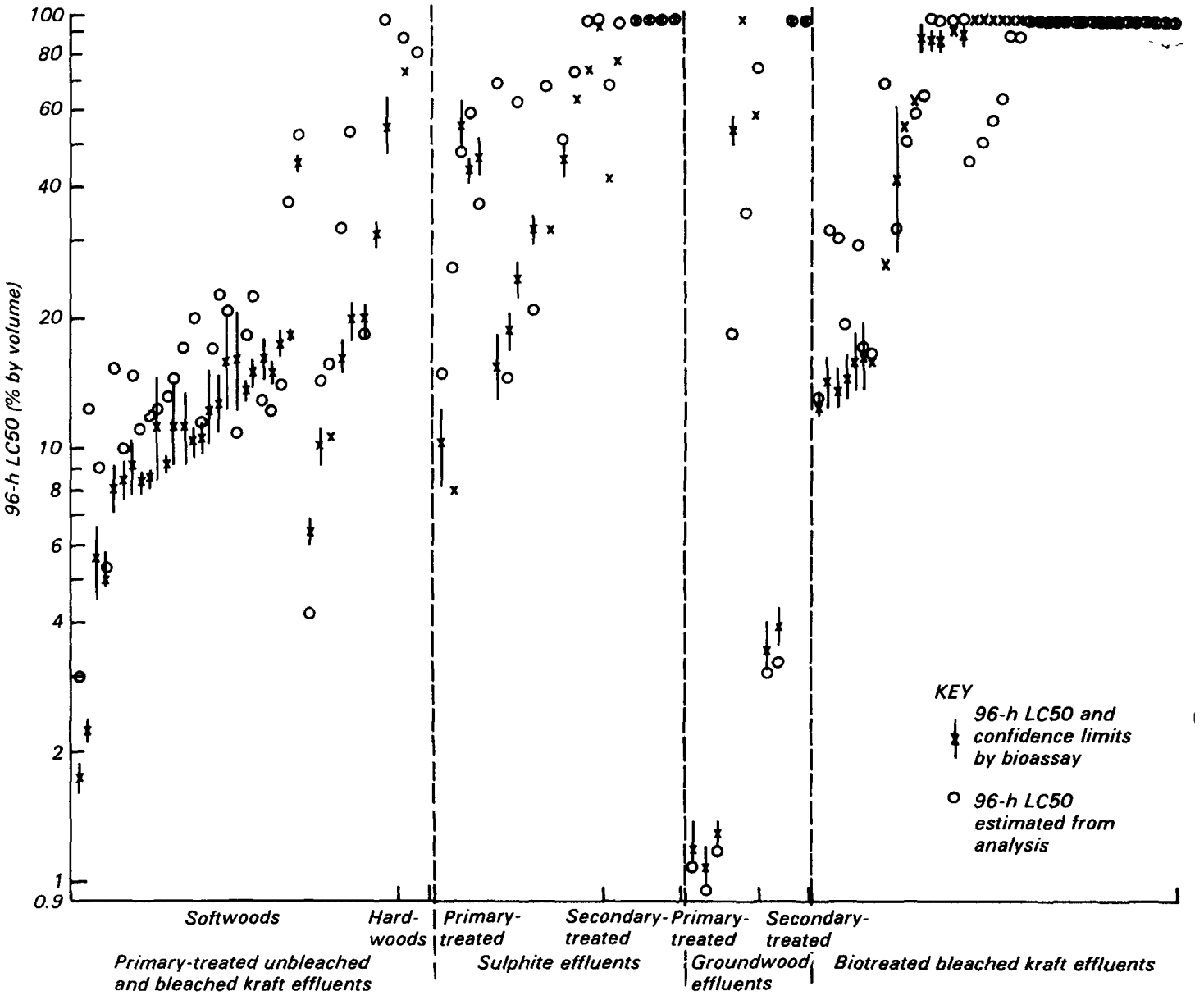

Figure 1. *Comparison of calculated and measured effluent toxicities.*

Recommendations

Reasons for the lack of agreement in some cases between calculated and measured toxicity should be investigated. As a first step, the toxic components should be identified in effluents from mills that use hemlock, cedar, or hardwoods.

Since most research in this area to date has been carried out with cold water fish species, a similar study comparing calculated and measured toxicity using warm water test fish species would be of relevance and value to mills in the south.

Consideration should be given to using "standard" 96-h LC50 values of the individual toxicants to calculate the toxicity of effluents to fish species. This would avoid problems of variations in bioassay results caused by the effects of seasonal and genetic differences on sensitivity between different stocks of the same species.

This study was concerned with acute lethal toxicity. Pulp mill effluents cause detectable sublethal effects in fish in the laboratory at concentrations of 0.1-0.2 of the 96-h LC50. Studies should be undertaken to determine the degree of effluent treatment necessary to ensure that concentrations of toxicants in receiving waters are below these levels.

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The complete report, entitled "Development of a Chemical Toxicity Assay for Pulp Mill Effluents," (Order No. PB 81-126 369; Cost: $11.00, subject to change) will be available only from:
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