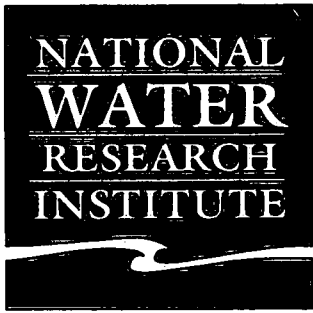
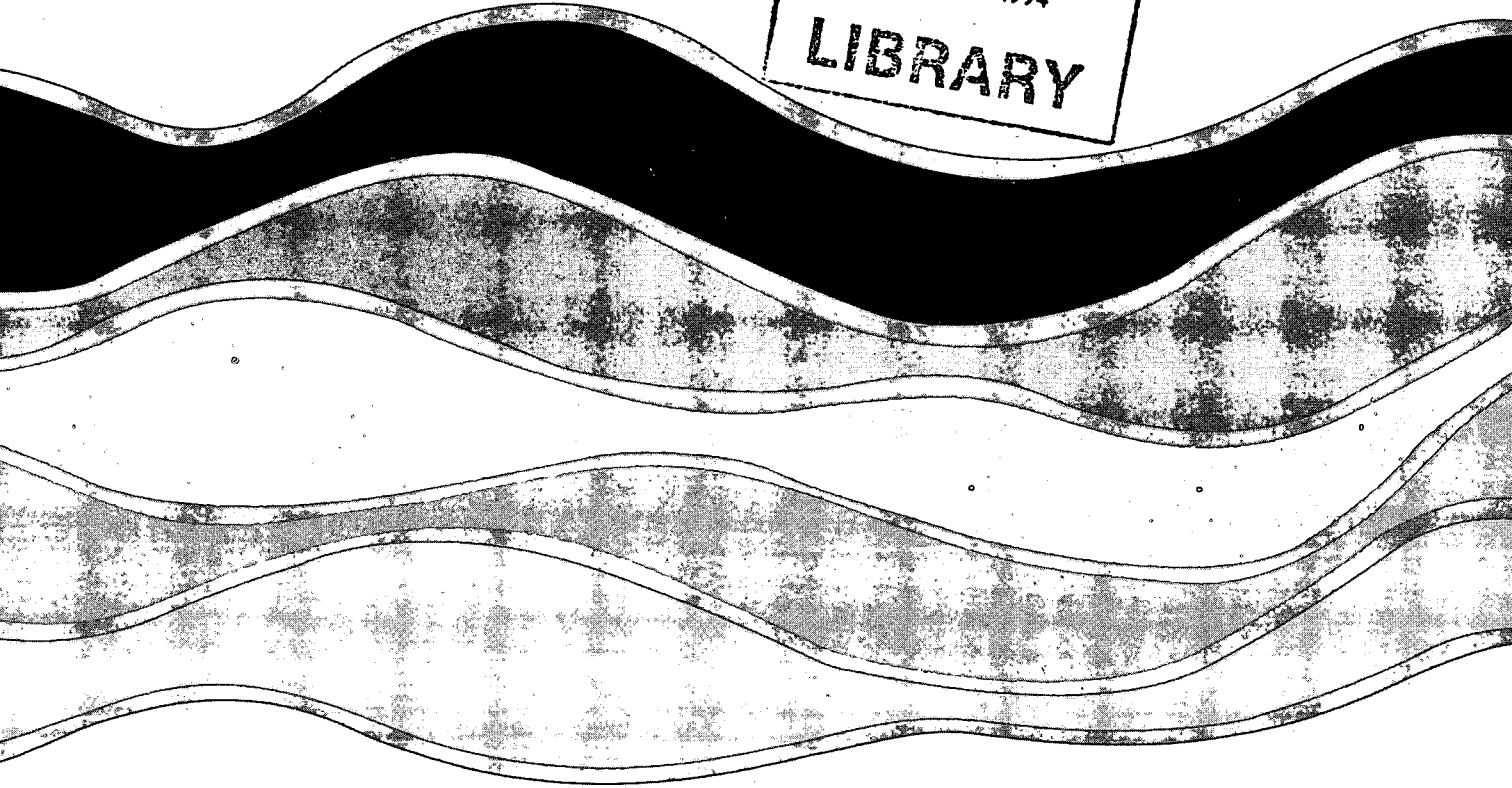


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**A PRELIMINARY CHEMICAL EXAMINATION OF
HYDROPHOBIC TIRE LEACHATE COMPONENTS**

PART I

**A COMPREHENSIVE ANALYTICAL APPROACH
TO THE IDENTIFICATION/CHARACTERIZATION
OF TIRE LEACHATE COMPONENTS**

D.H.J. Anthony

NWRI Contribution No. 93-76

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**Research and Applications Branch
National Water Research Institute
Burlington, Ontario L7R 4A6**

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SERIES CONTENTS

PART I

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PART II

**ON-SITE, LARGE-SAMPLE PRECONCENTRATION OF TIRE LEACHATE
COMPONENTS FOR TOXICITY-BASED CHEMICAL CHARACTERIZATION**

D.H.J. Anthony and D.W. Barclay

PART III

**PRELIMINARY CHROMATOGRAPHIC AND FTIR/UV/VIS
SPECTROMETRIC EXAMINATION OF MAJOR TIRE LEACHATE
COMPONENTS**

D.H.J. Anthony and A. Latawiec

MANAGEMENT PERSPECTIVE

This work forms part of a larger study within the Analytical Chemistry Research Project (ACRP)/Research and Applications Branch (RAB) to identify toxic components of tire leachate. The rationale for this larger study is to develop an analytical approach for the identification/characterization of unknown toxic contaminants in environmental aqueous systems. This approach is necessary in situations where toxicity of waters cannot be explained by results of "target" compound analyses. The unexplained toxicity of kraft pulp and paper mill effluents after removal of adsorbable organohalogen compounds (AOX) is an example of such a case. The approach suggested in this report would be of value to the mandate of the CEPA and sustainable development as the identification of toxic compounds and determination of their source allows a more rational basis for regulation than "blanket" regulations which tend to have a severe economic impact on selected industries.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Cet article est tiré d'une grande étude faite dans le cadre du projet de recherche en chimie analytique, Direction de la recherche pure et appliquée, dont l'objectif est l'identification de composés toxiques trouvés dans le lixiviat produit par l'entreposage des pneus. Ce qu'on cherchait à faire avec cette étude, c'était de mettre au point une méthode analytique d'identification et de caractérisation de contaminants toxiques inconnus qui se trouvent dans des systèmes aqueux dans l'environnement. Cette approche est rendue nécessaire par le fait que certains cas de toxicité de l'eau ne peuvent pas être expliqués à partir des résultats d'analyse de composés «cherchés». C'est le cas, par exemple, de la toxicité inexpliquée de la pâte kraft et des effluents des fabriques de pâte à papier après extraction des composés halogénés adsorbables (AOX). La méthode proposée dans cet article aurait son utilité dans le cadre de la LCPE et dans la perspective du développement durable, en ce que l'identification de composés toxiques et la détermination de leurs sources créeraient une base plus rationnelle pour la réglementation que ne le sont les règlements «généraux» qui ont généralement un effet économique grave sur des secteurs industriels déterminés.

ABSTRACT

This report describes problems encountered in explaining the toxicity of tire leachates and suggests an analytical approach which may be effective in achieving this goal. The integration of large-sample extraction (LSE) with various molecular spectrometric detection techniques is discussed.

RÉSUMÉ

Cet article décrit la difficulté d'expliquer la toxicité des lixiviats produits par l'entreposage des pneus; il propose aussi une méthode d'analyse qui peut se révéler efficace. Il est question de l'intégration de l'extraction sur de gros échantillons et de différentes techniques de détection spectrométrique au niveau moléculaire.

INTRODUCTION

Rubber chemistry is quite well understood in the sense that chemicals used in manufacture of the material and the final product are quite well characterized although the identity of many of these materials is proprietary (1). Not so well understood, or disclosed, is the nature of chemicals produced in the material as by-products of the manufacturing process or the degree to which they and various starting materials are leached from the material in water-based applications such as building harbour breakwaters or creating artificial reefs as fish habitat.

Several suggestions have been made, and some projects initiated locally (2), to explore the use of scrap tires as artificial reefs for fish habitat. Similar use has been made of this material elsewhere in North America for at least the last decade (3). Ironically, recent testing has shown that leachate of scrap tire material shows acute lethal toxicity to rainbow trout fry (4). Studies, using the US EPA's Toxicity Characteristics Leaching Process (TCLP) on shredded tire material (5), or similar toxicity testing procedures using whole tires have shown that tire leachates demonstrate toxicity in a number of biological test methods (3,4,6).

Tire Leachate Compounds - Failure of the Target Compound Approach

A toxicity study conducted under contract from NWRI by B.A.R. Environmental Inc. (4) has shown that tire leachate from both new and scrap tires is toxic to rainbow trout fry under standardized conditions of the 20-day static acute lethality bioassay. The scrap tire showed greater toxicity, reaching lethal levels in as little as 5 days. This toxicity persisted during the test period and beyond (40 days). Tire leachate samples from this testing have been analysed for target, and some non-routine organic contaminants using dichloromethane (DCM) extracts from the processing of small sample

volumes. These analyses (GC/MS, GC/AED) did not detect significant levels of target compounds. Problems with the precipitation of solid material during the analytical concentration process also complicated the analysis. Samples from this study were also analysed for toxic levels of "heavy metals" and other inorganic species with similar negative results.

These results suggest that the "culprit" organic or organometallic toxic components (i) are of unknown identity or undetected by conventional routine methodology, (ii) may be too hydrophilic for effective isolation by solvent extraction, or, (iii) may be highly toxic target or unknown contaminants exerting effects at ultratrace levels. It is evident from these results that a more comprehensive, "broad-spectrum" analytical approach is required for identification/characterization of the toxic component(s) (7).

As yet, the demonstrated toxicity of tire leachate material cannot be correlated to the presence of "target" compounds included in "Priority Substances" lists (eg. 8,9,10).

Most current environmental chemical analyses are applied to the determination of a restricted number of target contaminants. This approach, adopted by the US EPA in the mid-1970s for the "Superfund" project, was chosen for very practical and economical reasons in tackling a massive hazardous waste site cleanup problem (10). This effort focussed on compounds known to be extremely hazardous ("Priority Pollutants") and which could be determined with the most sensitive existing detectors, the electron capture detector (ECD) or the mass spectrometer (MS), in conjunction with gas chromatographic (GC) separation techniques. Little effort was allotted for research in the development of analytical methodology for determination of the remaining millions of known and unknown compounds likely to be present in environmental aqueous systems (11). This deficiency has been pointed out in recent years and a realization is developing as to how limited our vision is by focussing so exclusively on the determination of a relatively small number of target contaminants (12).

The target compound approach is suitable for the "crisis" type management required in the assessment and cleanup of hazardous waste sites and grossly polluted water systems. It is not sufficient for preventative and "early warning" monitoring and surveillance activities (13). Some environmental analytical laboratories are beginning to restructure to accommodate this realization. The Ontario Ministry of the Environment's Drinking Water Laboratory is making an effort to accommodate these concerns in designing a program to identify contaminants in water other than the routine "Priority Substances" (14).

A More Comprehensive Analytical Approach to Toxicity Studies

In past years, several researchers involved in analytical methods development for water quality monitoring purposes have proposed comprehensive "master" analytical fractionation schemes for the determination of "all" (ie. all that were then known) chemical components of an aqueous sample (15,16); others have proposed various fractionation procedures for specific chemical classes of components (17). Large-sample extraction has been applied to the determination of toxicity (Ames Test) in surface waters (18). A comprehensive toxicity-based fractionation/characterization approach of this sort has recently been proposed by Afghan (7).

Recent improvements in instrumental and methodological capability suggest that these earlier proposed schemes may be reinvestigated and expanded to provide a much more comprehensive determination of major, minor, trace, and ultratrace components of water than was possible even a decade earlier. An opportunity also exists to simplify some determinations which are presently being accomplished in a somewhat disjointed manner with separate samples being taken or processed for different types of analyses.

As a relatively uncomplicated example of these possibilities, advances made in preconcentration technology, such as the Goulden Large-Sample Extractor (GLSE)

(19-23) which allows isolation of hydrophobic organic contaminants from large (10-1000+ L) water samples, offer the potential for integration with instrumental analytical techniques which have been improved to a point where they may be used to advantage in the analysis of environmental aqueous samples (24). Such instrumental techniques include infrared spectrometry, resurrected as the more sensitive and less time-consuming Fourier Transform Infrared (FTIR) technique, atomic emission spectrometry, used with the microwave-induced plasma source, as a detector (AED) for elemental analyses (25) and nuclear magnetic resonance spectrometry (NMR).

The integration of chemical information from these and other (eg. GC/MS, GC/ECD, GC/FID, etc.) fundamentally different instrumental techniques can provide much more satisfying confirmation of compound identity for which spectral and chromatographic libraries exist and is essential in the identification or characterization of unknown compounds (26-28). Large-sample extraction provides a means by which sufficient material for this type of comprehensive analysis may be obtained.

Characterization and Identification of Organic Compounds by Molecular Spectrometry

Some classical molecular spectrometric instrumental techniques such as infrared (IR) spectrometry, UV/VIS spectrometry, NMR spectrometry and ESR spectrometry have seen little use in routine environmental analysis to date primarily because of sensitivity limitations. These techniques, however, offer valuable information which can be used in elucidation of the chemical structure (identity) of unknown compounds. They have been traditionally used, albeit at very high levels (% or neat) of the compounds of concern, in conjunction with other more sensitive molecular spectrometric techniques such as MS for the identification/characterization of unknown compounds.

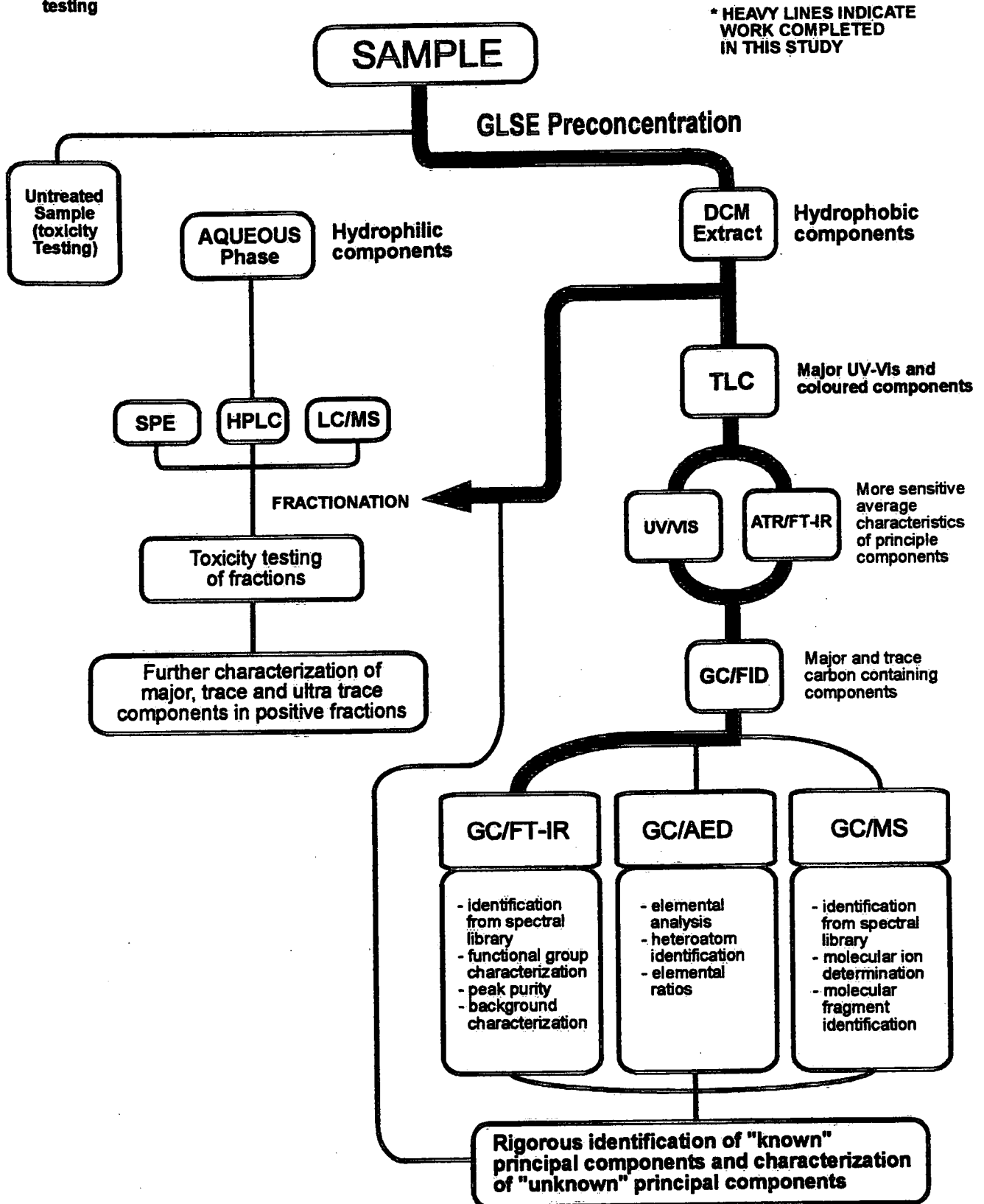
The more sensitive analytical techniques (GC/MS, GC/ECD) have been applied primarily in confirmation of the identity of a relatively small number of target

compounds for which mass spectral libraries and chromatographic retention times exist. Alone they cannot provide rigorous chemical identification of unknown compounds. These libraries appear extensive but, in reality, cannot keep pace with the thousands of new compounds appearing annually in industrial applications (28,29). Protection of ecosystem health requires a much more comprehensive information base of the ambient levels of known synthetic organic chemical compounds as well as possibly an equal number of unknown by-products of industrial (eg. petrochemical, P&P and agrochemical) process reactions and environmental degradation processes. Possibly the most well-known situation of this nature (unknown by-product) was the appearance of polychlorinated dibenzo-dioxins in the environment. These compounds were unintended and, for many years, undetected by-products of the synthesis of pentachlorophenol, used in the formulation of wood preservative materials. It is comforting perhaps, but unwise, to think that this class of compounds is the only one of its sort in the environment. It is likely that many such compounds exist and exert toxicity, both acute and chronic, at trace levels.

Rigorous identification of known and unknown organic chemical contaminants requires more information than can be obtained by reliance on the application of a single separation/detection technique (eg. GC/MS, GC/ECD). Information from GC/MS analysis, however, can be integrated with that provided by other, fundamentally different, structure elucidation techniques (eg. AED, FTIR, NMR, ESR) to provide a rigorous confirmation of known compounds or to provide identification or, at least, a chemical characterization of unknown compound(s) (27-29). This information, in conjunction with quantitative information on ambient levels and corresponding toxicological information, would allow assessment of the impact of the compound(s) on aquatic ecosystem health and may provide the basis for regulatory action.

The large preconcentration factors which can be achieved with the GLSE continuous-flow solvent extraction technique allows advantage to be taken of these informative, but less sensitive, techniques in the chemical characterization, identification and quantitation of unknown contaminants in aquatic systems (24). Fig. 1 shows an

Fig. 1 An analytical scheme for comprehensive water analysis (major and trace components) integrated with toxicity testing



example of how information from such techniques might be integrated in the chemical characterization of unknown compounds. In this approach, both the aqueous and organic extract phases are examined by various instrumental structure elucidation techniques after fractionation (GC, LC) to isolate individual components. Information from the structure elucidation techniques (molecular spectrometric techniques) is then integrated to suggest the identity of the components or at least indicate their functional characteristics. The latter information alone may be sufficient for regulatory purposes (the "standardless" approach (28)).

Advances recently made in the area of infrared spectrometry, with the development of Fourier transform instrumentation, brings this technique closer to application in trace and ultratrace determination of organic contaminants in aqueous matrices. The Fourier transform technique introduces a significant improvement both in the sensitivity and the speed of analysis over earlier dispersive IR measurements.

Experiments in the integration of large-sample extraction with UV/VIS and FTIR spectrometry for identification of unknown hydrophobic organic components of the tire leachate matrix were conducted and are described in **Part III** of this report series.

Large-Sample Preconcentration and Toxicity Testing

These non-routine applications of GLSE have previously been suggested by the author (24). Lippincott *et al* have recently demonstrated the use of large-sample extraction (LSE) in an ultrasensitive Ames test procedure (18). The advantage of this preconcentration technique is that it can provide sufficient material for the numerous chemical fractionations required for toxicity testing (7, 30-32) and for application of less-sensitive, but more informative, structure elucidation techniques for identification/characterization of toxic components. Two approaches to the identification/characterization of tire leachates were considered. One approach focusses on identifying, or at least characterizing, all detectable components of the sample and assessing toxicity

on the basis of known toxicity of these compounds, with consideration given to possible cumulative and synergistic effects. If the toxicity cannot be explained, then chemical fractionation, followed by toxicity testing of fractions and characterization of components in toxic fractions is done. A second approach, which might be called a "blind" fractionation, would chemically fractionate the sample and test the toxicity of each of the fractions, then apply chemical characterization/identification procedures only to those fractions showing positive toxicity. This approach is somewhat optimistic in expecting that the toxicity can be explained by the presence of a single component. In this study, a combination of the above procedures was used with an initial characterization/identification and toxicity assessment being made of major components before fractionation for toxicity testing. This strategy minimizes some of the disadvantages of the two approaches suggested in that it provides knowledge of principal components which may be used as markers (reference points) during subsequent fractionation. The results can also be more informative, by virtue of the characterization/identification done on all detected components, in determination of the impact of detected components on aquatic ecosystem health. Negative toxicity results obtained using any particular organism, or even through the "battery of tests" procedure, do not necessarily mean a compound has no effect on aquatic health (31). Toxicity tests applied to date on tire leachate samples are described in (3,4,6).

Molecular Spectrometric Examination of Tire Leachate Components

FTIR Spectrometry

Infrared spectrometry has been used for decades as a basic instrumental technique in the structure elucidation of organic compounds. IR spectrometry offers information on the types of functional groups in a compound (saturated hydrocarbon, olefinic, hydroxyl, amine, carbonyl, etc.). The Fourier transform (FT) technique, applied to IR spectrometry, provides many advantages over conventional dispersive IR measurements, in which absorption is examined through an optically created frequency

spectrum (dispersion by diffraction grating or prism). In the FT technique, spectral information (frequency and intensity) is instead collected in the time domain and then converted to the frequency domain by application of the mathematical Fourier Transform. The time domain information is created by collecting the IR radiation in a manner in which constructive and destructive interference of all frequencies in the radiation is allowed to occur in specific, consistent manner (a cosine function).

A main advantage of FTIR over the dispersive technology is that of the speed with which information is collected. With the FTIR technique, all information necessary can be collected in a second as compared to 15-30 minutes for the dispersive technique. The information is collected with the same sensitivity as dispersive measurements and is easily improved by repetitive scanning and co-adding of spectra to "average-out" random noise. Sensitivity in the FTIR technique is further improved by the greater light throughput allowed as slits are not needed to control resolution. All wavelengths are automatically calibrated during sampling with a HeNe laser. Stray light is of no concern in FTIR spectrometry, nor is sample emission. Sample heat-up is minimal. The FT sampling technique allows advantage to be taken of powerful data processing techniques such as sample/blank ratioing, background subtraction, interactive spectral subtraction, background characterization, spectral ratioing and rapid reference library searching.

These improvements to IR spectrometric measurements bring FTIR technique into the region where it can be of use in environmental analyses. This is particularly so with the GC/FTIR technique when used in conjunction with large-sample preconcentration of analytes from aqueous matrices.

GC/FTIR

The interfacing of GC and FTIR spectrometry is an excellent application of FTIR as the sampling involved, as a result of GC separation, is generally that of pure

compounds. The interface consists of a low dead-volume "light tube" through which the GC effluent passes and IR sampling is done "end-on" through this tube so that each compound eluting from the column can be viewed for several seconds allowing repetitive IR scans to be made (Fig. 2). The light-tube and associated GC transfer line equipment are temperature controlled to accommodate characteristics of the GC separation. The resolution capabilities of capillary GC are necessary for this application in order that only one compound be in the light-tube at one time.

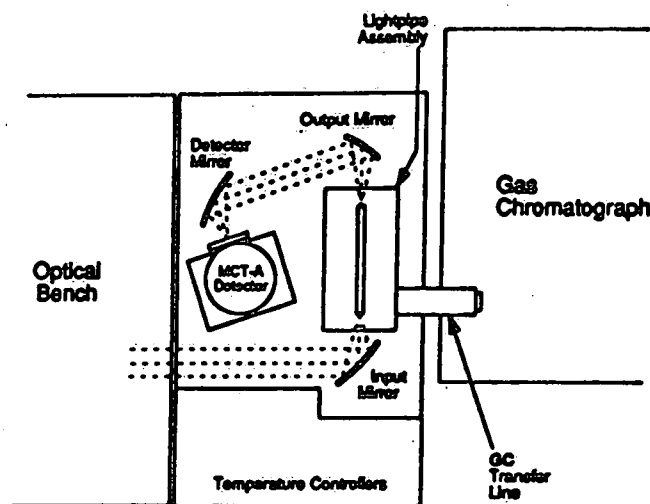


Fig. 2. GC/FTIR Interface (Diagram from Nicolet Instrument Co. material, with permission Nicolet Instrument Canada, Inc.)

"Off-the-shelf" detection limits for this technique, used under standard operating conditions, are between those of the FID (flame ionization detector) and the TCD (thermal conductivity detector), in the order of ng/L, dependent on the sensitivity of the most IR sensitive functional group in the compound studied. With large-sample preconcentration, response can be improved by factors of 10 - 1000 (24). In addition to the functional group characterization capability of the IR technique, its characteristic "fingerprinting" ability, derived from intramolecular effects on bond deformations, proves very useful in distinguishing between isomers which elute chromatographically (GC) with

poor resolution or unresolved (28). This feature can be utilized in complementing the MS technique and has been used, for example, to differentiate among isomers of chlorophenols, Mirex, DDT, chlordane, dieldrin, endrin, BHC, endosulfan, PCBs, PCDDs/PCDFs, PAHs and nitro-PAHs (28,32). An example of the power of the functional group characterization capability of the GC/FTIR technique is given by a demonstration that the carcinogenicity of Mirex may not be due to the parent compound but to a contaminant (triphenylphosphine oxide, a known carcinogen) to which the ECD was insufficiently sensitive (32). This example represents one of possibly many similar cases in which conclusions are drawn from the use of one type of detection specific to a restricted class of compounds.

A major advantage of the FTIR technique is the non-destructive operation of the detector. It may be integrated in GC and LC separational systems with a variety of other, destructive, detection systems (MS, ECD, FID, AED, etc.) to form part of a powerful integrated multidimensional, multidetection system valuable for the characterization/identification of unknown organic contaminants. A first step has been taken commercially in this direction by introduction of the GC/FTIR-MS (Hewlett-Packard) and the matrix-isolated GC/MI-FTIR (Mattson Instruments, (33)). This instrumentation, however, is still oriented towards the confirmation of identity of target compounds. Further integration with isolation, separational, and complementary structure elucidation techniques is necessary for application to the characterization and identification of contaminants other than those included in spectral libraries. Preliminary results of the application of this approach to the identification/characterization of tire leachate components are given in Parts II and III of this series.

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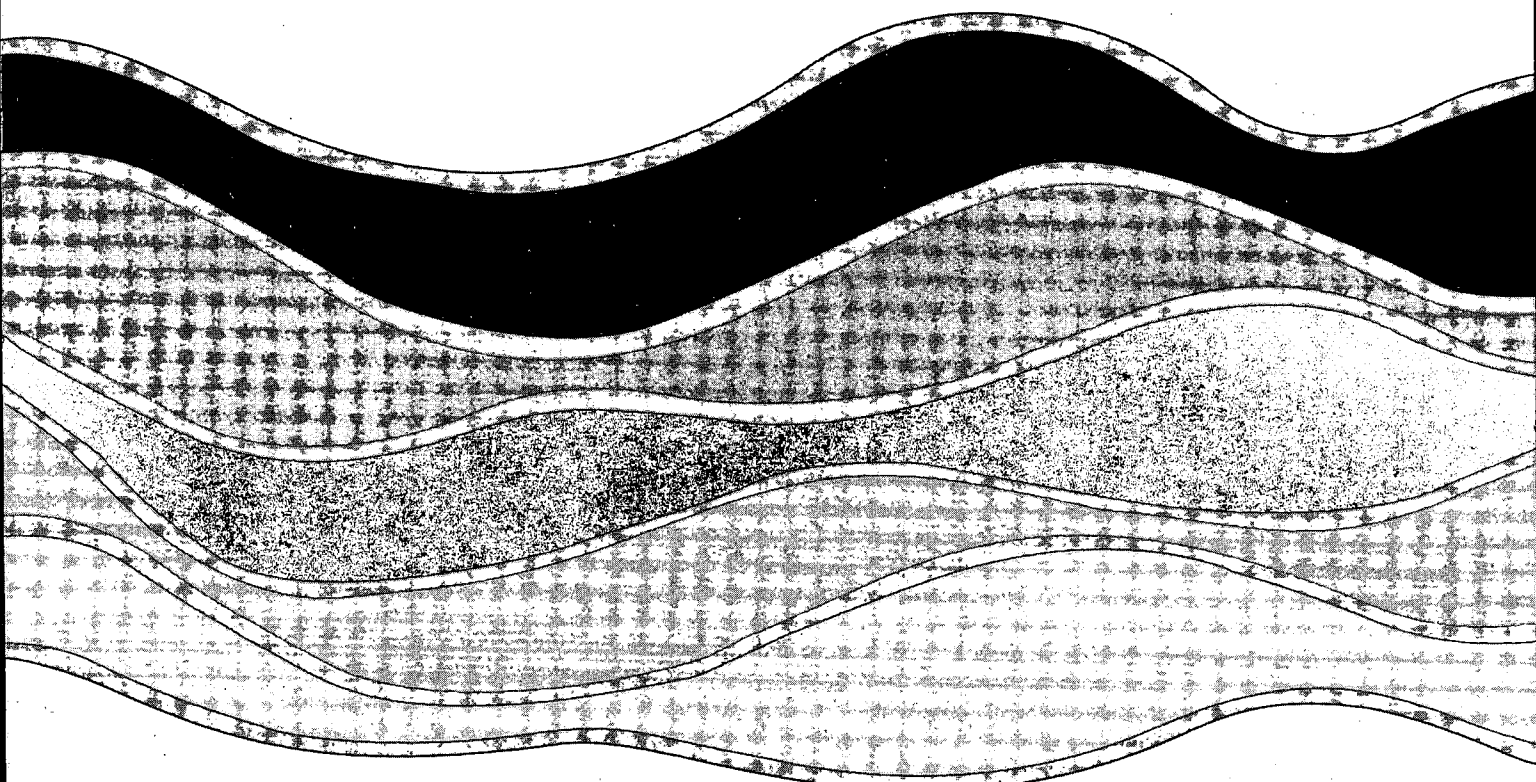
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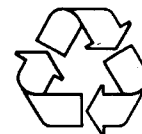
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