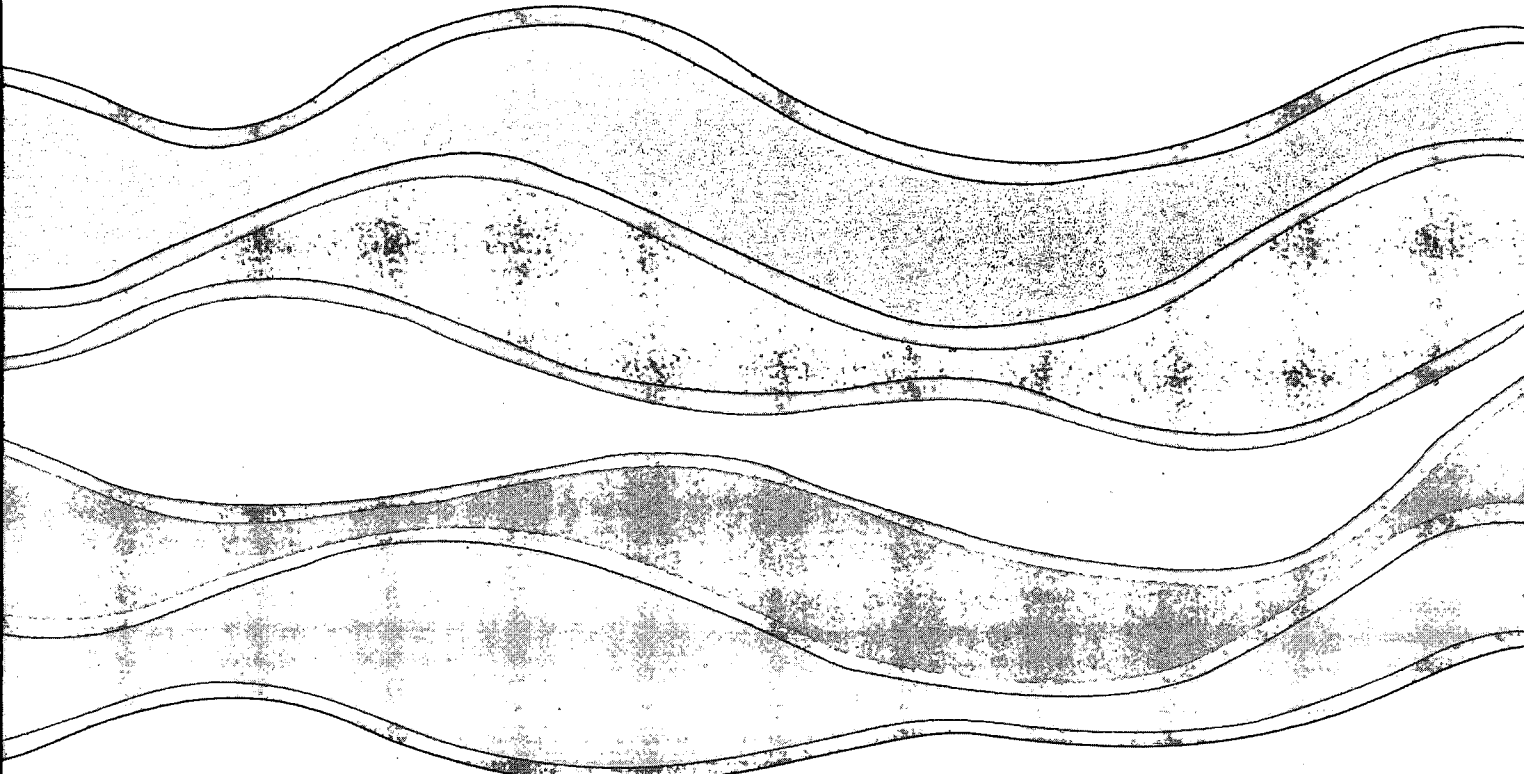


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**A SPECTROMETRIC AND  
CHROMATOGRAPHIC CHEMICAL  
COMPARISON OF SOLVENT EXTRACTS OF  
WHOLE TIRE LEACHATE AND OF  
SHREDDED TIRE LEACHATES OBTAINED  
AT VARYING SALINITY**

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## MANAGEMENT PERSPECTIVE

Used tires are contemplated for applications involving long-term immersion in aquatic ecosystems including the international waters of the Great Lakes. Tire leachates have shown acute toxicity towards several aquatic organisms, including rainbow trout fry. The Ontario Ministry of the Environment and Energy (OMEE) has placed a moratorium on the use of tires in such applications until a better knowledge of the characteristics of tire leachate compounds is had (toxicity, bioaccumulation, persistence). The Maryland Department of Natural Resources (MDNR) is investigating toxic characteristics of tire leachates in order to make decisions regarding the application of used tires as artificial aquatic reef habitat in Chesapeake Bay. As part of an ongoing project within the RAB / NWRI studying toxic organic contaminants in tire leachates, a cooperative study was undertaken with the MDNR to compare principal organic contaminant patterns in leachates from whole tires and from shredded tire material. The latter material has been proposed for use as a "worst-case scenario" test for aquatic re-uses of used tires and is being used by the MDNR in toxicity testing.

In this work, solvent extracts of shredded- and whole-tire material showed the presence of similar compounds although this may not be generally true for all types of whole-tire material. Some of the principle compounds detected have been tentatively identified by GC/FTIR but further substantiation of identity by other structure elucidation techniques (eg. GC/MS, GC/AED, HPLC/MS, etc.) is required. Materials detected are spread through a broad range of chemical polarity indicating that GC, LC, and SFC separation and detection techniques may be required in isolation of the toxic components. Both leachate generation techniques provide similar amounts of extractable material but both need to be scaled up to provide sufficient material for comprehensive toxicity-based chemical characterization of leachate compounds. Large-sample extraction, solvent fractionation and preparative scale LC techniques are required for isolation and preliminary fractionation of these compounds. Shredded-tire material displays high levels of toxic metals which can complicate the determination of toxic organic components. The results of this work augment those of previous studies in showing more clearly that the "target

compound" approach (attempting to relate toxicity to the presence of "priority substances" such as those included in the CEPA PSL) is inadequate in explaining the observed toxicity of tire leachates. A more comprehensive, "broad-spectrum" approach is needed in which toxicity testing is integrated with chemical testing in a manner so as to focus on toxic components for more exhaustive comprehensive chemical identification (toxicity-based chemical characterization).

## SOMMAIRE À L'INTENTION DE LA DIRECTION

On envisage d'immerger à long terme des pneus usés dans des écosystèmes aquatiques, notamment dans les eaux internationales des Grands Lacs. Il a été démontré que les lixiviats de pneus avaient une toxicité aiguë à l'égard de plusieurs organismes aquatiques, dont les alevins de la truite arc-en-ciel. Le ministère de l'Environnement et de l'Énergie de l'Ontario a imposé un moratoire sur ce type d'utilisation des pneus jusqu'à ce que nous disposions d'une meilleure connaissance des caractéristiques des composés présents dans les lixiviats de pneus (toxicité, bioaccumulation, persistance). Le Maryland Department of Natural Resources (MDNR) étudie actuellement les caractéristiques toxiques des lixiviats de pneus afin de prendre des décisions concernant l'utilisation des pneus usés comme habitat aquatique récifal artificiel dans la baie Chesapeake. Dans le cadre d'un projet en cours à la Direction de la recherche pure et appliquée de l'INRE sur les contaminants organiques toxiques dans les lixiviats de pneus, on a entrepris avec le MDNR une étude conjointe visant à comparer les principaux types de contaminants organiques dans les lixiviats de pneus entiers et déchiquetés. Comme les pneus déchiquetés présentent le plus de risques, on a proposé qu'ils soient utilisés dans les tests concernant les réutilisations aquatiques des pneus usés, et le MDNR utilise actuellement ce type de matériel dans ses tests de toxicité.

Dans la présente étude, les extraits par solvant de pneus entiers et de pneus déchiquetés ont révélé la présence de composés similaires, mais il se pourrait que ce ne soit pas toujours le cas avec tous les types de pneus entiers. On a essayé d'identifier certains des principaux composés détectés par couplage entre la chromatographie en phase gazeuse et la spectroscopie infrarouge à transformée de Fourier, mais pour mieux connaître l'identité des substances, il faudra recourir à d'autres techniques d'élucidation des structures (p. ex. CPG/SM, CPG/DEA, chromatographie liquide à haute performance/SM, etc.). Les substances détectées montrent une vaste gamme de polarité chimique, ce qui indique que les techniques de séparation et de détection par CPG, CPL et CPS peuvent être requises pour l'isolement des composants toxiques. Les deux techniques de génération des lixiviats fournissent des quantités similaires de matière extractible, mais les deux doivent être améliorées pour fournir suffisamment de matière pour une caractérisation chimique détaillée

axée sur la toxicité des composés présents dans les lixiviats. L'extraction de gros échantillons, le fractionnement par solvant et des techniques préparatives de chromatographie liquide sont requises pour l'isolement et le fractionnement préliminaire de ces composés. Les pneus déchiquetés renferment de fortes concentrations de métaux toxiques, ce qui peut compliquer la détermination des composants organiques toxiques. Les résultats de cette étude enrichissent ceux d'études précédentes en ceci qu'ils montrent plus clairement que l'approche fondée sur des composés cibles (qui tente de relier la toxicité à la présence de substances d'intérêt prioritaire comme celles incluses dans la liste des substances interdites de la LCPE) ne permet pas d'expliquer la toxicité observée des lixiviats de pneus. On a besoin d'une approche plus globale à large spectre dans laquelle les tests de toxicité se trouvent intégrés aux tests chimiques d'une manière qui permette de se concentrer sur les composants toxiques en vue d'une identification chimique plus détaillée et exhaustive (caractérisation chimique axée sur la toxicité).

## ABSTRACT

The pattern of organic contaminants leached from whole and shredded used tires has been compared by GC/FTIR, GC/MSD, UV/VIS-PDA spectrometry and by TLC after initial polarity fractionation by solvent extraction. The comparison shows the patterns leached from both matrices are similar and that the extracted material does exhibit positive results in the Microtox test. Shredded tire material, however, shows very high concentrations of zinc, iron and manganese. This may complicate determination of the effects of leachable toxic organic contaminants. The results show as well that the amounts of extractable material recovered in both test methods are comparable but that for a comprehensive chemical examination, scale-up of the "standard" test methods is required. Organic contaminants identified and/or characterized in the tire leachate extracts by GC/FTIR are reported.

## RÉSUMÉ

Les types de contaminants organiques lixiviés tirés de pneus usés entiers et déchiquetés ont été comparés par CPG/spectroscopie infrarouge à transformée de Fourier, CPG/DSM, UV/RVS-PDA et chromatographie sur couche mince après avoir d'abord séparé les contaminants en fonction de leur polarité au moyen d'une extraction par solvant. La comparaison montre que les types de contaminants obtenus des deux matrices sont similaires, et que le matériel extrait donne des résultats positifs dans le test Microtox. Cependant, les pneus déchiquetés renferment de très fortes concentrations de zinc, de fer et de manganèse. Cela peut compliquer la détermination des effets des contaminants organiques toxiques lixiviables. Les résultats montrent aussi que les quantités de matière extractible obtenues dans les deux méthodes d'essai sont comparables, mais que les méthodes d'essai «standard» doivent être améliorées pour permettre un examen chimique plus détaillé. On présente les contaminants organiques identifiés et (ou) caractérisés dans les extraits de lixiviat de pneus par CG/spectroscopie infrarouge à transformée de Fourier.



## **INTRODUCTION**

The re-use of used tires is of environmental importance because of the large quantity of existing material presently in storage (1), and because of the many re-use applications being examined. Whole tires may be used as artificial reefs for fish and shellfish habitat, dock, canal, and boat "bumpers", breakwaters (2), and building materials. In the past, however, most whole tire material in Ontario has been disposed of as landfill. Presently it is being stored in open "tire dumps" as the practices of landfill use and incineration of tire material are no longer environmentally acceptable in Ontario (1). Shredded tire material is used in production of asphalt, construction materials, rubber mats, etc.(3)). U.S. federal regulations require the use of shredded tire material in asphalt production. A relatively large-scale local application of shredded tire material (114,000 tires) as a "builder" in the construction of a highway overpass embankment has recently been proposed (4). Ultimately, all of these re-uses involve exposure of tire material to water by immersion or by weathering due to precipitation. The resulting leachate components, and environmental degradation products of parent components, can eventually reach surface and/or groundwater systems.

Toxicity of tire leachate to aquatic organisms has been reported (5,6,7,8). A recent electron microscopy study of tissue effects in sheepshead minnow larvae resulting from exposure to tire leachates suggests involvement of a neurotoxin(s) (9). Identification of toxic, persistent and bioaccumulative tire leachate components is in only the early stage of development. The Ontario Ministry of the Environment and Energy (OMEE) has recently placed a moratorium

on re-use of used tires in aquatic applications until more information is obtained on compounds leached from this material and their environmental impact (10).

The objective of the work described in this report was to determine whether whole-tire leaching and shredded-tire leaching (by a modified Toxicity Characterization Leaching Procedure (TCLP) (11)) are comparable in terms of the types and amounts of chemical compounds appearing in leachate. The latter point is of importance as a rather large amount of leachate components must be collected to conduct a comprehensive chemical examination and toxicity-based chemical characterization of leachate components (12,13). As well, the comparison was undertaken to determine if the leachate component patterns showed any differences which might be attributed to surface reactions of the whole tire in interaction with pavement during normal use. It is theorized that the high-temperature, high-pressure interaction of tires with pavement (particularly asphalt) might lead to the production of toxic oxidized chemical species (14).

## **EXPERIMENTAL**

In previous work with dichloromethane (DCM) extracts of whole-tire leachate the observation was made that a precipitate often appeared on evaporative concentration of extracts solvent-exchanged into isooctane and that this precipitate was readily redissolved in DCM (15,16). The reconstituted concentrates gave GC/FTIR chromatograms identical to those of the original DCM concentrates. The isooctane concentrates, on filtration, were seen to contain most

of the gas chromatographable material while the DCM concentrates contained some gas chromatographable components as well as non-gas chromatographable components (as visualized by TLC). This observation suggested that in examining tire leachates, an initial solvent fractionation might be useful in sorting leachate components into broad chemical classes. Such fractionation might simplify subsequent chemical characterization and toxicity assessment. The solvents required for this process have been shown to be easily removed from methanol concentrates to the point at which they do not affect the Microtox screening test (16)). Microtox testing was applied to extracts resulting from solvent fractionation to locate gross toxicity characteristics which could be further separated by appropriate techniques (GC, HPLC, SFC).

Whole tire leachate was produced as previously described (6) and processed by solvent extraction using the Goulden Large-Sample Extraction (GLSE) technique (17). The technique was applied in a manner so as to isolate a greater fraction of hydrophilic compounds (18). Leachate components were isolated in 800 mL DCM extracts resulting from processing of 100 L of each leachate sample (control, used-, new-tire). Extracts were further concentrated by evaporation and solvent exchanged into 100 mL of methanol (1000-fold overall concentration factor) (18,19) for spectrometric and chromatographic examination and toxicity testing (12,13,15,16).

Smaller samples ( $\approx 3$  L) of leachate from shredded tires were obtained at varying salinities (0, 5, 25 ppt (parts per thousand)) (Table I) using a modified version of the US EPA TCLP (11). A mixture of tire chips was used, with the same relative proportion of tire types as found in a typical storage pile, and included truck, car, white wall, black wall, steel-belted and bias-ply material. Tires were shredded mechanically then reduced in size using hand tools to conform with TCLP extraction specifications ( $\approx 1 \text{ cm}^3$ ). A total of 140 gm tire chips / 2.8 L was used for each salinity level, corresponding to a leachable surface area of  $\approx 330 \text{ cm}^2 / \text{L}$ . This material was tumbled in salt water for 7 days at 2 rpm then leachate was filtered through 37 and 10/m mesh, settled overnight and decanted.

The shredded tire leachate samples were processed at NWRI as received without further filtration (Table I). A selective extraction procedure was used to fractionate components into major chemical polarity / ionic classes (non-polar, moderately polar, acidic, basic, strongly polar) (Fig. 1). Preconcentrations with isooctane were done in a "batch" manner using 1 L Teflon separatory funnels. Dichloromethane extractions were done using the "Mini-GLSE" technique (20). In both cases, three successive extractions were used. Single acidic and alkaline extractions were done employing the "Mini-GLSE" with DCM as solvent. Demixing extractions with isopropanol (IPA) (21) were done in the 4 L glass containers used for sample collection. Evaporative concentration of the extracts was accomplished using a Zymark Model 'TurboVap' Evaporator. Isooctane was evaporated at  $40^\circ \text{C}$  with a dry nitrogen (UHP) stream at 150 KPa; DCM was evaporated at room temperature with the same gas pressure. Evaporations were

stopped when the liquid level was 1-2 mL and liquid was quantitatively transferred to graduated 15 mL Pyrex centrifuge tubes. The liquid (concentrate plus rinsings) was then blown down, at water bath temperatures (40-50 °C) where necessary, with a nitrogen stream to provide concentrates with an overall concentration factor ( $\times 1000$ ) equivalent to that used for "stock" concentrates in the study of whole tire leachate (15). The stock concentrates were further evaporated to volumes suitable for particular analyses (Table I). The concentration scheme was designed so that at no point would the concentrates need to be evaporated to dryness to exchange solvents. In the study of whole tire leachates, the latter transfer technique was made necessary as the concentrates had been prepared in methanol for HPLC analyses (13,19).

Isopropanol extracts were not analyzed by the techniques described below as the matrix is not suitable for GC work using non-polar bonded phase (DB-5). These were retained for developmental work in LC/FTIR characterization which is the subject of another study.

### **Metals Analyses**

After acidification to 0.2 % HNO<sub>3</sub> (Baker, 'Ultrex' conc. reagent), metals analyses were conducted by the National Laboratory for Environmental Testing (NLET) using the ICAP-direct aspiration schema (22).

### **Organics Analyses**

UV/VIS-PDA, GC/FTIR, and GC/MSD spectrometry and TLC of the extracts were conducted following adjustment to final volumes representing equivalent concentration factors for determination of absolute differences between samples (Table I). The application of these techniques has been described previously (14,15).

### **Extractable Residue**

After chemical analysis, concentrates were diluted to 4 mL, and 1 mL of each concentrate was evaporated to "dryness" on 51.5 mm diameter watchglasses (the term "dryness" is an operational definition used in this study determined by a definite point of inflection in a drying curve distinguishing evaporation of solvent from volatilization of analyte). Residue was weighed using a Sartorius model 1712 MP8 analytical balance.

### **Toxicity Testing**

Microtox toxicity testing (22,24) of solvent fractions was conducted as previously described (16). Concentrate volumes were adjusted to allow comparison with Microtox data for the whole-tire experiment.

## **RESULTS / DISCUSSION**

The results of a chemical examination of whole tire leachates have been reported (15,16). This section describes results obtained from examination of shredded tire leachate and a comparison of features of the two tire leachate extract types.

### **Characteristics of Un-processed Shredded Tire Leachate**

All shredded tire leachate samples were clear and colourless. Colour in the "0 ppt" leachate was associated with particulate / flocculent material which settled on standing. Other physical parameters are shown in Table I.

UV/VIS-PDA spectrometry of the unprocessed shredded tire leachate samples showed a strong absorbance at 200 nm even in the 25 ppt control sample. This absorbance was attributed to the presence of iodide which is a minor constituent in most synthetic sea salts such as that used in this study to adjust salinity. This control sample was thus used as the reference to be subtracted from the other leachate spectra. Un-processed aqueous phase leachate samples showed minimal absorbance features with this correction.

### **Characteristics of Processed (Solvent Extraction) Leachates**

Solvent extracts of shredded tire leachate samples, obtained by sequential solvent extraction (Fig.1), were evaporated to a common small volume (4 mL). They were lightly

coloured with the DCM and acidic extracts showing the most intense colouration (orange-brown). UV/VIS-PDA spectra (Fig. 2) obtained in DCM show broad absorbance features, with the 25 ppt salinity leachate concentrates showing the broadest absorption bands. Specific isolated bands or distinct shoulders were observed in the 25 ppt leachate isooctane concentrate (450 nm) and in the 5 ppt basic concentrate (442 nm). The 25 ppt control concentrates all showed narrow absorbances limited to < 300 nm. These results, on comparison with those seen for the unprocessed aqueous samples (above) suggest that UV/VIS-PDA spectrometry, with a relatively small preconcentration factor (x 10-100) might be used as a convenient means of following tire leachate kinetics.

#### Non-Polar Components (Isooctane extracts)

By GC/FTIR analysis, isooctane extraction was seen to isolate a number of compounds from the leachate samples including benzothiazole, 2-morpholiniothio benzothiazole, a long-chain unsaturated ketone (Rt 5.56 min) and the "nitro-1,-2" compounds observed in the previous experiment with whole tires (15,16). The 5 ppt isooctane concentrate showed, in addition, a series of saturated hydrocarbons. GC/FTIR chromatograms with identifications and characterizations are shown in Figs. 3a-c. GC/FTIR repon data are given in Table II. GC/MSD total ion chromatograms supported the FTIR assignments of some of these compounds but the absence of a spectral library for this technique precluded identification or characterization of other compounds (Fig 4a). GC/MSD analysis was conducted on the 0 ppt salinity leachate extract only, as this contained all the compounds seen in the higher salinity extracts. Differences in



sensitivity between the FTIR and MSD detection techniques were apparent, some compounds giving better FTIR response than MSD response and vice versa. TLC showed the presence of coloured compounds between  $R_f$  0.3 and 0.7. Only a small amount of hydrophilic material ( $R_f < 0.3$ ) was observed, suggesting that these extracts were enriched in hydrophobic, presumably gas chromatographable, material (Fig. 5). Some spots above  $R_f$  0.4 showed long-wave UV absorbance and fluorescence. Aging of TL chromatograms resulted in the appearance of an intense orange-brown spot ( $R_f \approx 0.2$ ) for all salinities.

#### Moderately Polar Components (DCM extract)

GC/FTIR analysis of the DCM (neutral pH) extracts showed that benzothiazole, 2-morpholiniothio benzothiazole and the long-chain unsaturated ketone compounds are effectively extracted in the exhaustive isooctane extractions. The DCM extracts contained a larger amount ( $\approx 2X$ ) of the "nitro" compounds isolated in the isooctane extractions and comparable to levels seen in DCM extraction of whole tire material (15). As with the whole tire study, these compounds appear to make up the bulk of gas-chromatographable material (80-90 %) extracted by DCM. The DCM extracts also contained appreciable amounts of a phthalate ester (tentatively identified as bis-2-ethylhexyl phthalate). This compound may be a contamination artifact (possibly plastic bottle caps exposed to solvent/water mixture). Additional compounds tentatively identified in DCM extracts include cyclohexene oxide and 2-chloro cyclohexanol (likely derived from cyclohexene oxide). These compounds are likely oxidized derivatives of cyclohexene, used in the preservation of reagent DCM. A branched alcohol was also characterized as were some additional

nitro compounds appearing to contain OH and C=O functionalities. A very broad peak possibly a triazine derivative, was seen in the 25 ppth DCM extract (Figs. 3a-c, Table II). GC/MSD confirmed the identity or functional character of some principal components but others were seen at levels too low to obtain useful mass spectra (Fig. 4b).

TLC of the DCM concentrates from subsequent extraction of aqueous phases of the isooctane extractions showed broad bands of coloured material ranging from  $R_f$  0.0 to 0.7. A preponderance of more hydrophilic, non-gas chromatographable compounds with  $R_f$  values less than 0.3 were noted with an intense spot remaining at the origin for all salinities. Long-wave UV and fluorescent spots were noted in the chromatogram (Fig. 5). Ageing of the chromatogram showed an intense purple spot at  $R_f$  0.25. The colour changes noted in some spots in TL chromatography of the isooctane and DCM extracts was similar to that observed in TLC and LC fractions of whole tire DCM extract (15,16).

#### Acidic Compounds (Acidic extraction)

GC/FTIR analysis of DCM extracts from acidic extraction of the leachate samples showed only a phthalate (possibly bis- ethylhexyl), a small group of branched carboxylic acid compounds ( $R_t$  5.75-5.95 min) that were poorly resolved and an unidentified lactone (Fig. 3a-c, Table II). TLC of the 5 ppth salinity extract showed only a faint diffuse coloured band of low  $R_f$  with an intense spot at the origin.

### Basic Compounds (Alkaline Extraction)

DCM extracts from alkaline extraction of the leachate samples showed, by GC/FTIR, a small amount of a branched amine ( $R_t$  11.95 min) (Fig. 3a-c, Table II). The 25 ppt control sample contained approximately 10-15 alkyl phthalate or alkyl phosphate derivatives and was the only extract to show these compounds. These were likely an experimental artefact resulting from exposure of sample to plastic material. TLC of the 5 ppt salinity extract showed a faint diffuse coloured area between the origin and  $R_f$  0.7 with some material remaining at the origin and two very narrow distinct spots at  $R_f$  0.1.

### Extractable Residue

For each salinity, the neutral pH DCM extraction isolated more material than each of the other three extraction techniques, and 50-60 % of the total residue recovered. For each salinity, the combined isooctane and DCM extracts contained 70-80 % of the total residue recovered. The amounts of DCM-extractable material recovered suggests that the majority of extractable leachate components are more hydrophilic in nature. More material (total) was collected in the saline extracts than the "0" ppt extract, supporting the above conjecture. The acidic extractions showed recoveries similar to the neutral pH isooctane extraction; the basic extractions showed minimal recoveries considering weighing error (Table III). The total residue weight, summed from solvent fraction residue weights, in each of the shredded tire leachates was less than recovered in the whole-tire leachate (15,18) but still of a comparable magnitude. This seems a contradiction with the estimated leachable surface areas for the two test materials (Table III) and may be related to

the estimate for shredded tire material which is probably far greater, due to its porosity, than given by a simple geometric calculation. The vigour of the TCLP process may also account for the amount of extractable residue collected from the shredded tire material.

### Metals

Whole tire leachate showed typical background levels of metals, well below those thought to cause adverse effects in aquatic organisms. The shredded tire leachates showed very high levels of zinc, iron and manganese (Table IV). Zinc, at such levels, has been implicated as the major toxicant in a recent study of tire leachate generated from plugs of tire material (7). Zinc and iron concentrations showed an inverse relationship to increasing salinity; this may be an analytical artefact related to the effect of high levels of "major ions" (Na, K, Ca, Mg) on ICP responses for trace metals (28,29). Iron and manganese, in the ratio observed in this work, likely result from steel tire belting or as an artefact from abrasion of tools used to shred the tires. Zinc, however, was found in this study and in (7) at high levels not explained by contamination associated with ferromanganese materials. Zinc forms, both inorganic and organic, are commonly used in tire manufacture formulations (26). High levels of zinc seen in leachates of shredded or cut tire material may result from exposure of such chemicals in the inner tire matrix to the leaching process. Alternatively, this may be due to manufacturing differences in tire brands. In whole tire studies (12,15,25) (eg. Table IV), observed metals concentrations are too low to explain leachate toxicity and the toxicant(s) are, therefore, assumed to be organic compounds. Toxicity of shredded tire leachate to sheepshead minnow larvae does not appear to involve morphological

features typical of metals contamination (9). The high content of alkali metals and alkaline earths seen in the shredded tire leachate tests at 5 and 25 ppt salinity are associated with the sea salt used to adjust salinity of the experiments.

### Toxicity Testing

Microtox toxicity testing (23,24) of the four solvent fractions of the 0 ppt salinity shredded tire leachate showed most toxicity to be present in the DCM extracts with a very high positive result for the acidic DCM extraction (Table V). Some toxicity was isolated in the isooctane extract and virtually none in the alkaline DCM extract. This further substantiates the conclusion that a single-stage DCM large-sample extraction with high solvent/water ratio (SWR), as was used in GLSE extraction of whole tire leachate (18), is effective in isolating toxic organic components of tire leachate as it recovers compounds of a broad range of polarity including the range effectively recovered by isooctane (15). Acidic and alkaline extractions with the GLSE apparatus are easily accomplished (20,27). These results also substantiate the conclusion that Microtox toxicity is expressed over a range of polarity (16). Subsequent fractionation of the components of the DCM extract into polarity classes by chromatographic means is facile.

Microtox toxicity in the shredded tire leachates has been shown to decrease with increasing salinity (8). GC/FTIR analyses showed a few unidentified but partially characterized peaks the intensities of which showed an inverse relationship with salinity, implying a possible positive relation to Microtox toxicity. Morpholiniothio benzothiazole also shows this relationship

(Table II). The latter compound, however, has not been implied in toxicity to rainbow trout fry (15). Benzothiazole, and the unidentified nitro compounds showed opposite behaviour, increasing in concentration with decreasing toxicity. This suggests that these principal leachate components are not likely involved in Microtox toxicity. It remains to be determined, however, if they are involved in acute toxicity to rainbow trout fry. Previous work has shown that morpholiniothio benzothiazole and the nitro compounds do not appear in tire leachate fractions, generated by classical LC, showing Microtox toxicity. The nitro compounds however did show a positive relation in toxicity to rainbow trout fry (16).

The high Microtox toxicity result seen for the acidic DCM extract is unexplained. Several unidentified compounds were noted in the GC/MSD TI chromatogram. Bis(2-ethylhexyl)-phthalate, two unidentified carboxylic acids and an unidentified lactone were the dominant compound noted in GC/FTIR. TLC showed only material at the origin, implicating very hydrophilic compounds.

## CONCLUSIONS

The results of this study may not be generally applicable to leaching characteristics of tire material. The comparison was done using only one whole tire and one sample of mixed shredded tire material. Considering the large number of compounds used interchangeably in tire manufacture (26), differences observed in this comparison may, in fact, be only that of differences in manufacturers or within product lines of particular manufacturers. The similarities noted, however, in GC and TLC chromatographable patterns observed for the two tire material types studied suggest that certain aspects of leaching patterns may be common to most tire material and may possibly be used as indicators of contamination of aquatic systems by tire material. GC/FTIR and GC/MSD chromatograms of the whole tire leachate and of shredded tire leachates were very similar in the sense that the number of principal components (trace levels; mg/L, /g/L) observed was relatively small, and several compounds were common to both leachate types. Ultratrace analysis (ng/L, pg/L) would undoubtedly increase the complexity of the problem but it seems appropriate to scrutinize major components before proceeding with such a project.

Morpholinothio benzothiazole and unidentified nitro-compounds formed dominant gas chromatographic features in both leachate types. The more hydrophilic compounds, as separated by TLC, showed similar patterns of coloured spots as well as some long-wave UV absorbing and fluorescent areas. These latter compounds, being non-gas chromatographable on non-polar phase columns, require examination by LC techniques. They comprise a significant proportion of observed Microtox toxicity in the whole-tire leachates.

Work with whole tires has shown that DCM-extractable, leached organic compounds do show toxicity in the Microtox screening test for acute toxicity. This toxicity is spread through a range of non-polar (gas chromatographable on non-polar phases) and more polar hydrophilic compounds (visualized by TLC). Shredded tire leachate shows similar extractable principal components and a similar broad range of toxicity among extractable organic contaminants. Microtox toxicity in this study, however, does not seem to correspond well with rainbow trout fry toxicity. This is not surprising considering the orders of magnitude differences seen in comparisons of Microtox toxicity, shown by specific compounds, with that expressed by other aquatic organisms. The trace components detected in this study should be fractionated by LC or preparative GC techniques (the latter is feasible with non-destructive GC/FTIR detection), whether identified or only characterized, and tested for toxicity by more rigorous tests than the Microtox screening test.

The solvent fractionation technique used in this study proved useful in further establishing that Microtox toxicity is not due to a single compound but to several compounds spread through a range of polarity. This rudimentary separation process simplifies gas chromatography of compounds by selective distribution among several fractions, thereby reducing co-elution phenomena encountered in GC of gross DCM extracts. The same advantage would apply to liquid chromatographic (eg. HPLC) separations.



Metals, although implied in tire leachate toxicity in other studies using shredded or cut tire material, do not appear to be involved in toxicity associated with whole-tire leaching. The Microtox toxicity observed in this study with shredded tire material and in previous work with whole-tires is due solely to DCM-extractable organic compounds. Rainbow trout fry toxicity, in the absence of significant metals levels, was observed in the whole-tire study. The presence of high levels of certain metals in leachate of shredded tire material may complicate assessment of toxicity due to organic contaminants in the aqueous phase through either direct or synergistic relationships.

Extractable residue weights determined in this study, and TLC, confirm earlier work suggesting that the bulk of extractable material is found in polarity fractions which, for the most part, are more amenable to analysis by HPLC. This is possibly not surprising as acute toxicity might be more readily expressed by low molecular weight, water-soluble compounds as these would be more likely to be rapidly incorporated into physiological processes of aquatic organisms, producing an acute toxic response.

Toxicity-based characterization of toxic organic leachate contaminants, therefore, requires an integration of GC and LC separation and detection techniques, possibly extending to higher molecular weight compounds requiring separation by GPC (Gel Permeation Chromatography). This conclusion may be applied generally to the examination of aquatic matrices, particularly those in which observed toxicity is not well-explained by determined levels

of PSL (CEPA Priority Substances List) compounds. Owing to a focus on organochlorine contaminants, environmental analytical chemistry is currently suffering from a dearth of information on environmental contamination regarding contaminants not amenable to determination by conventional GC protocols. LC techniques (HPLC/multidetector, HPLC/MS, GPC) and alternative GC techniques (GC/FTIR) in particular, should see greater application in examining cases in which PSL levels do not explain observed contamination effects.

It is concluded that whole-tire leaching, with some scale-up, is a more appropriate process for studying organic leachate compounds as the whole-tire "state" is that which would most likely be used in aquatic construction projects. Also, toxicity-based chemical characterization should not be complicated by the presence of high levels of metals (unless this characteristic is related to specific tire manufacture formulations). Little outstanding difference at trace levels, other than that of metals content, is seen between leachate of a whole-tire and from shredded tire material. Although the use of shredded tire material is intended to represent a worst case scenario, it also represents a case which is improbable in aquatic construction projects (artificial fish habitat, breakwaters, etc.); whole-tires or tire sections are used for these purposes. Large-sample DCM extraction of whole tire leachates has been shown to be useful in effectively isolating all but the very polar trace components of the leachate. Examination of the leachates at ultratrace levels absolutely requires large-sample preconcentration in order to apply chemical techniques required in identification and chemical structure elucidation. The standard TCLP test procedure provides comparable amounts of extractable material but both procedures require scale-up to provide

sufficient material for comprehensive chemical studies necessary for identification / characterization of toxic leachate components. The problem of metals content leached from shredded tire material, as would be experienced in a scale-up of the TCLP procedure, could be addressed by toxicity testing of the aqueous phase before and after processing by ion-exchange or by other techniques for amelioration of the effects of metals (eg. chelating agents).

#### **ACKNOWLEDGEMENTS**

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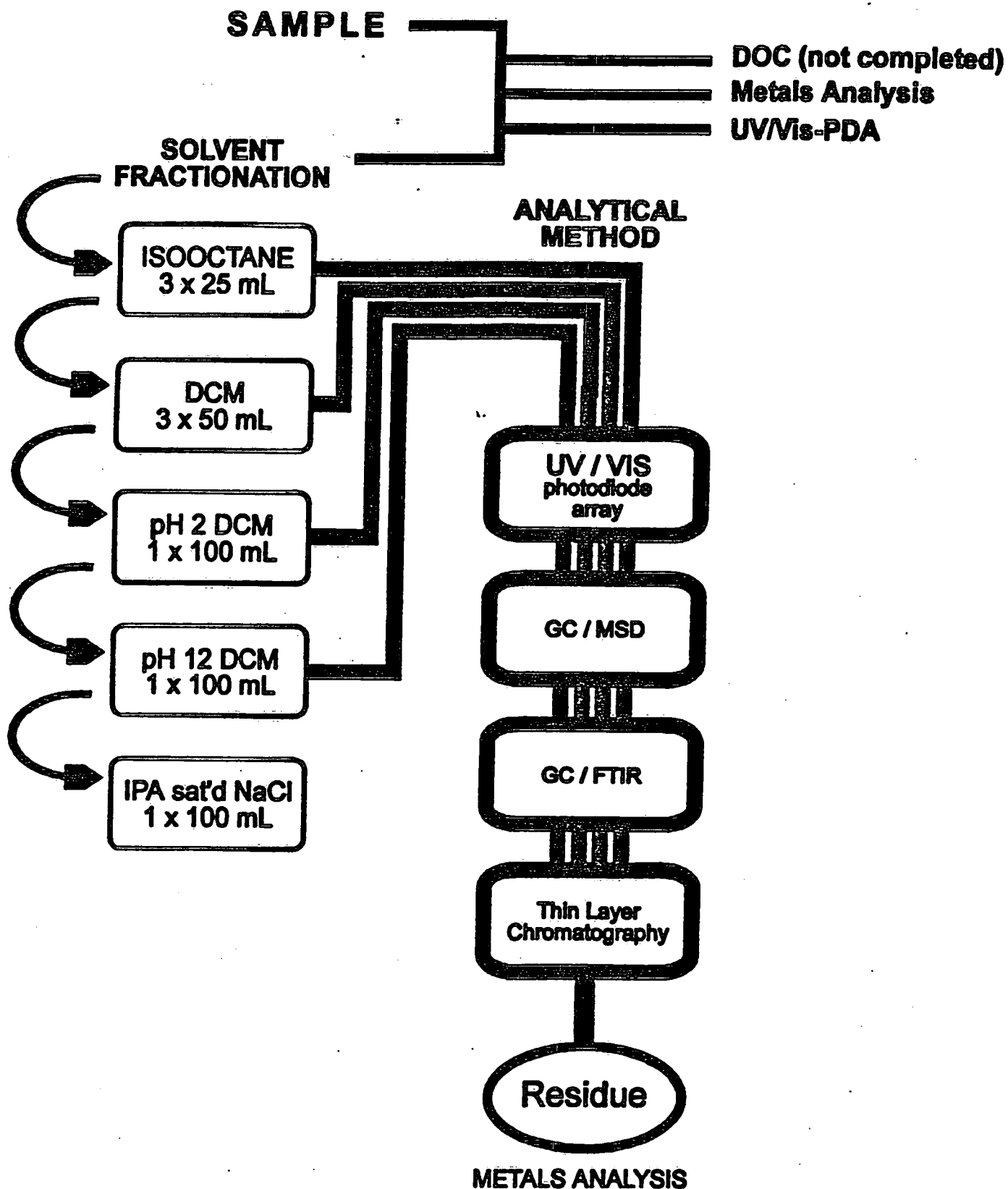
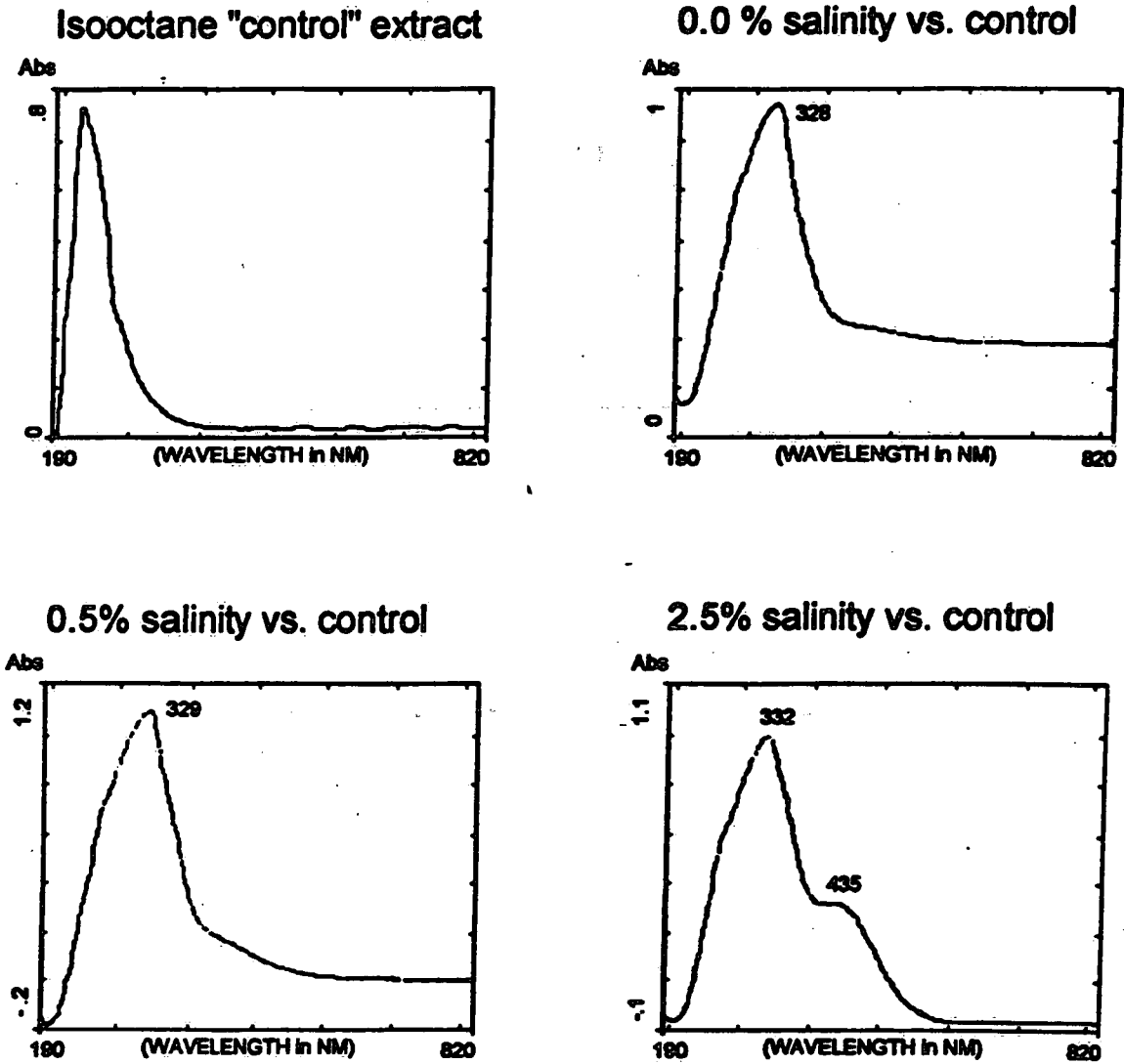
**Figure 1. Solvent Fractionation and Analytical Scheme**



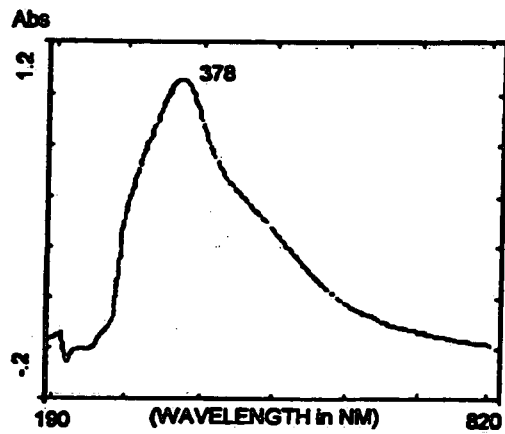
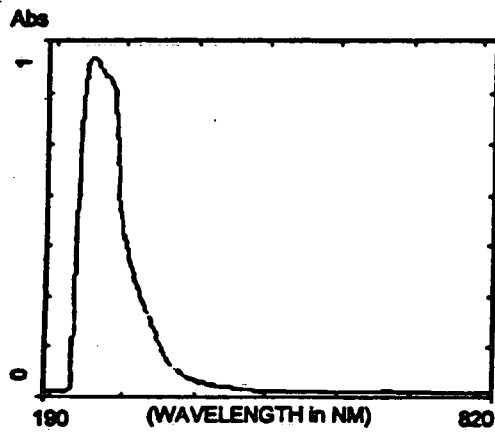
Figure 2A UV-Visible Spectra of Neutral isooctane extracts



## Figure 2B UV-Visible spectra of Neutral dichloromethane extracts

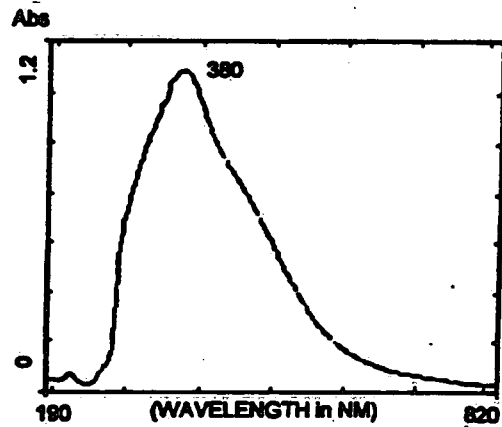
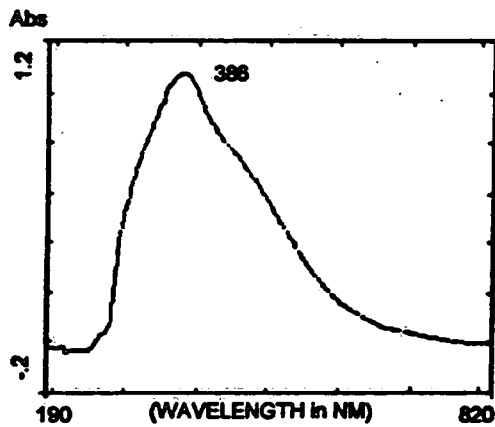
Dichloromethane "control" extract

0.0 % salinity vs. control



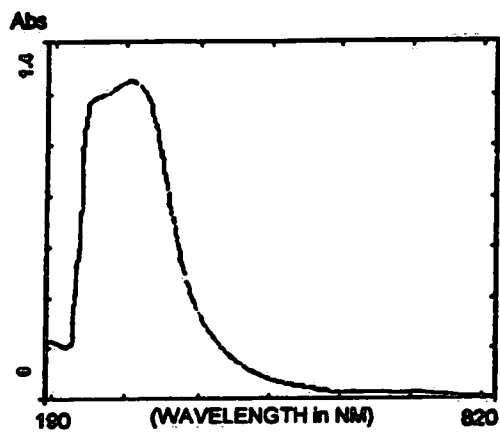
0.5% salinity vs. control

2.5% salinity vs. control

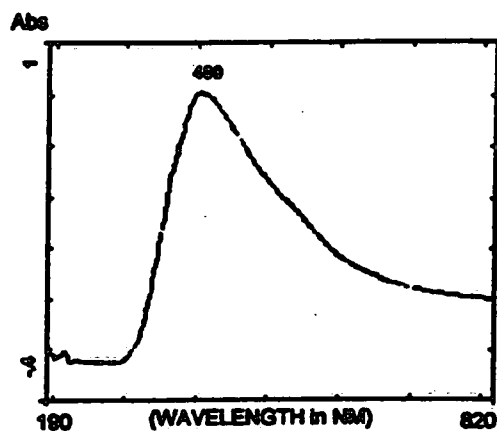


## Figure 2C UV-Visible spectra of Acidic dichloromethane extracts

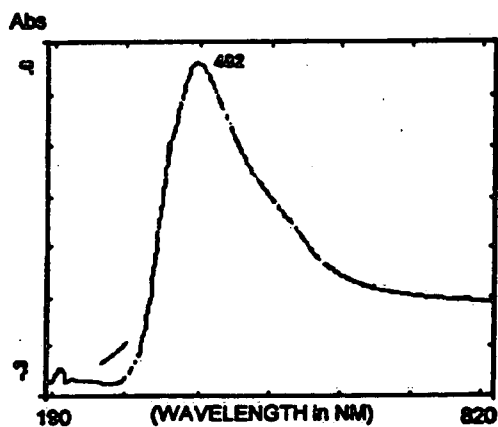
Dichloromethane "control" extract



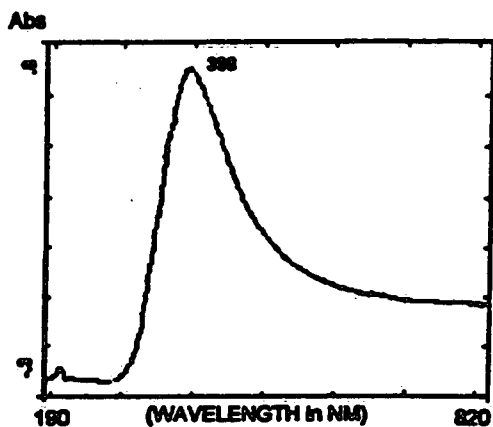
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0.5% salinity vs. control



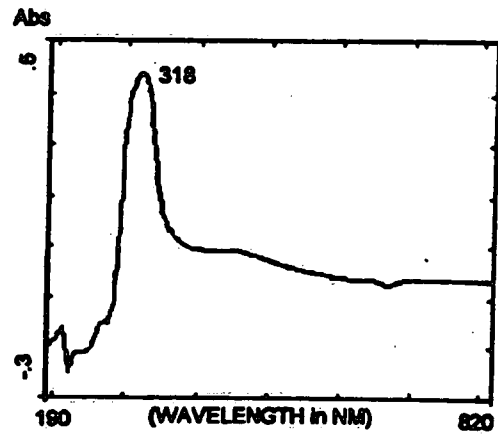
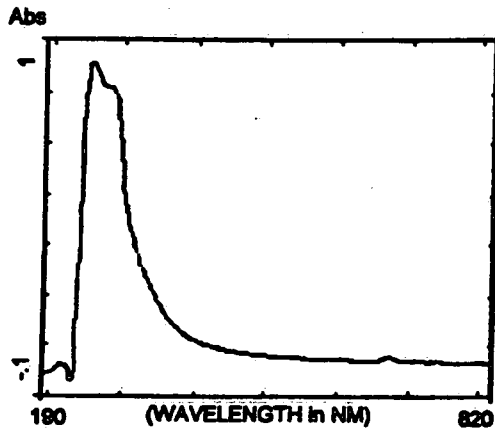
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## Figure 2D UV-Visible spectra of Alkaline dichloromethane extracts

Dichloromethane "control" extract

0.0 % salinity vs. control



0.5% salinity vs. control

2.5% salinity vs. control

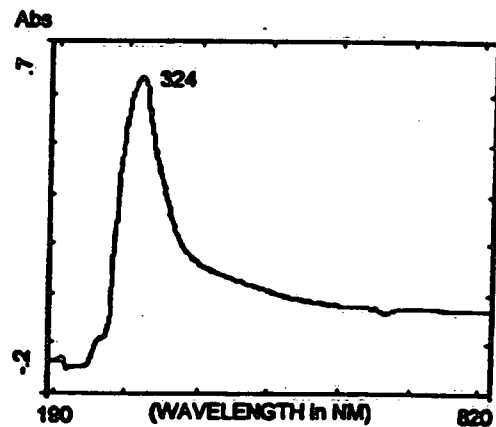
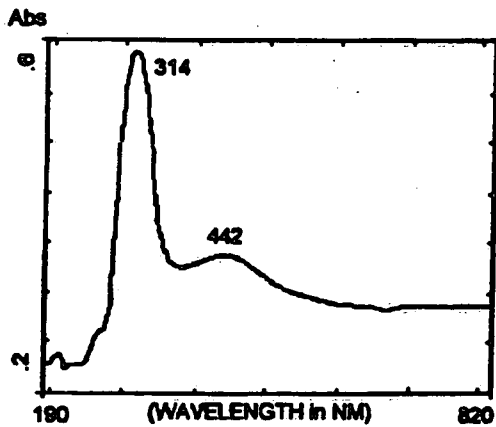
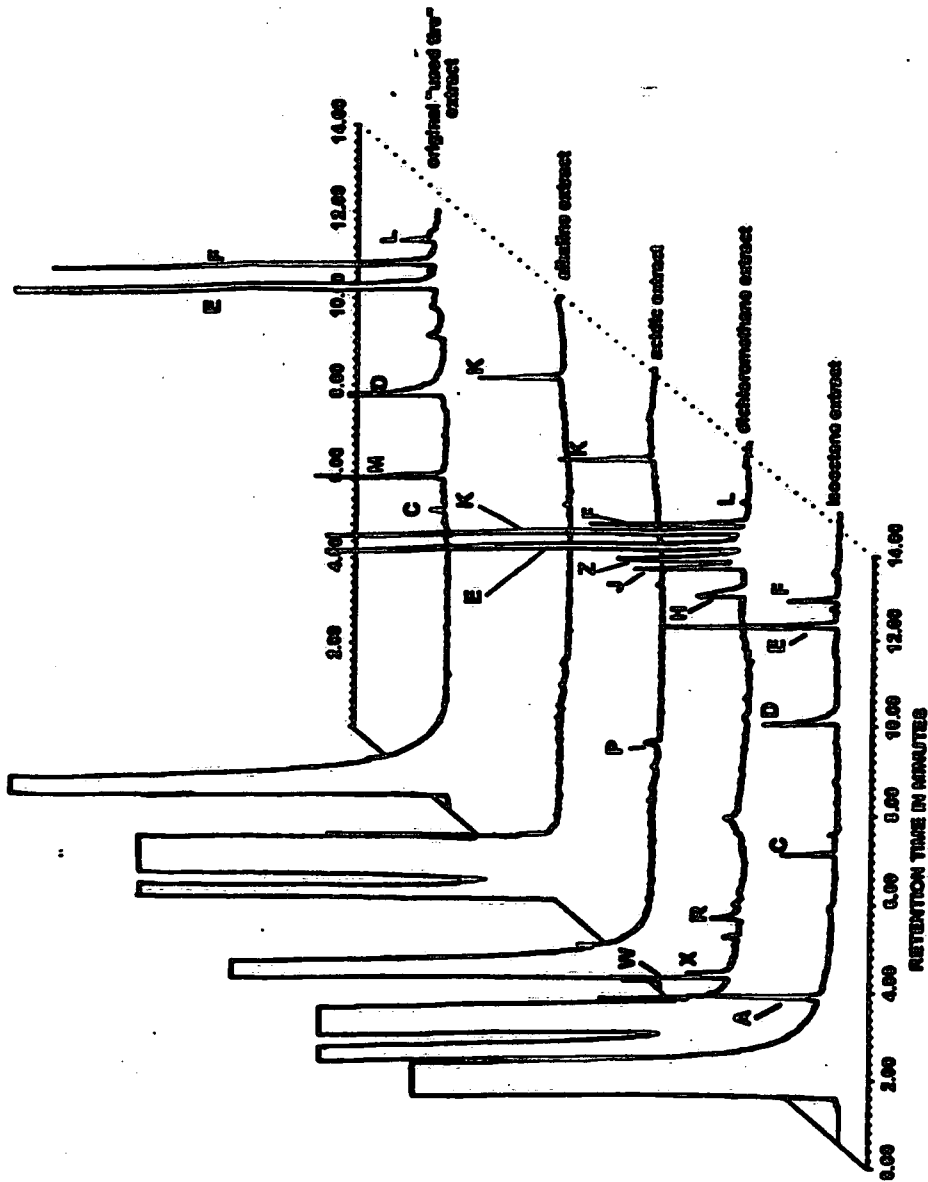
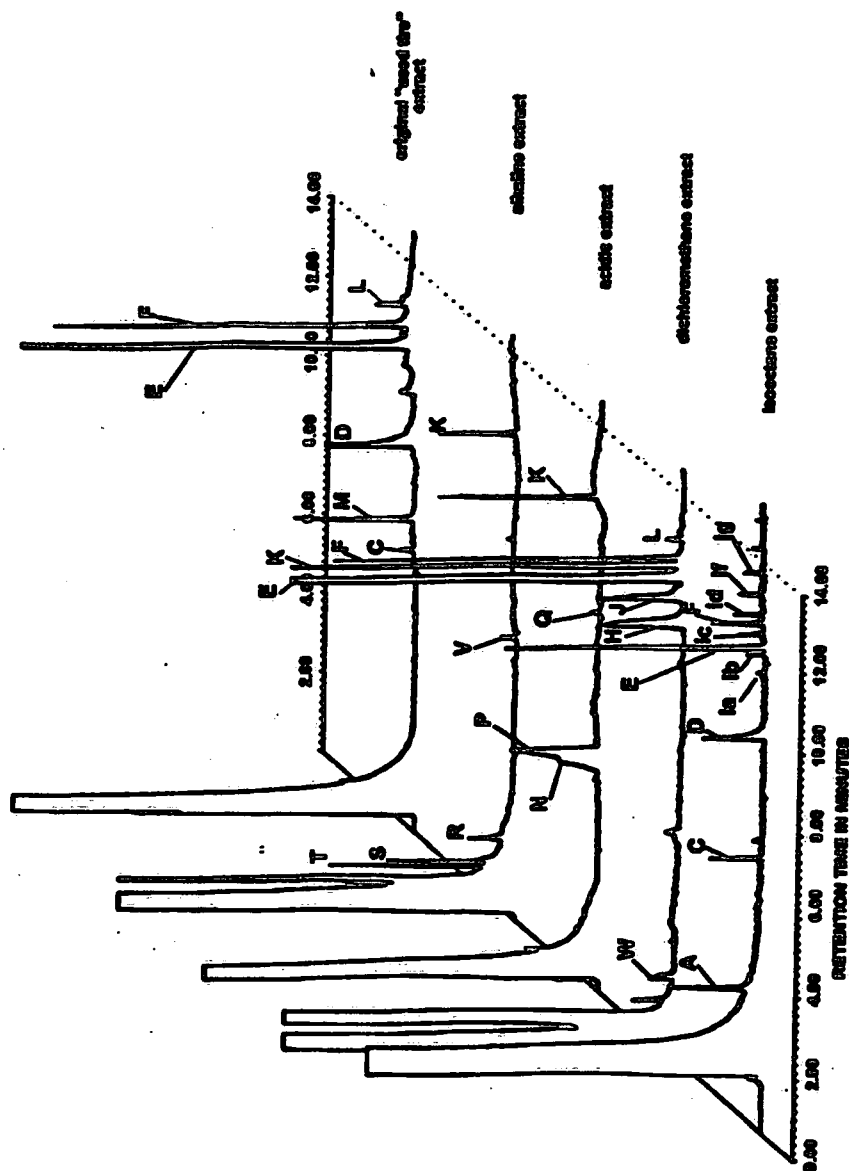


Figure 3A Comparative FTIR chromatograms of 0% salinity leachates



- Most Probable Identification**
- A 2° or 3° alcohol
  - C Unsaturated ketone
  - D Morpholinothio benzothiazole
  - E Nitro 1
  - F Nitro 2
  - G triazine der. ?
  - H Nitro 1A
  - J Nitro -OH
  - K phthalate (ethylhexyl)
  - L Nitro 3
  - M Alkyl ether der.
  - P Carboxylic acid (2,2-dimethyl der.)
  - R 2-chlorocyclohexanol
  - W Cyclohexene oxide
  - X Alcohol
  - Z Nitro / carbonyl

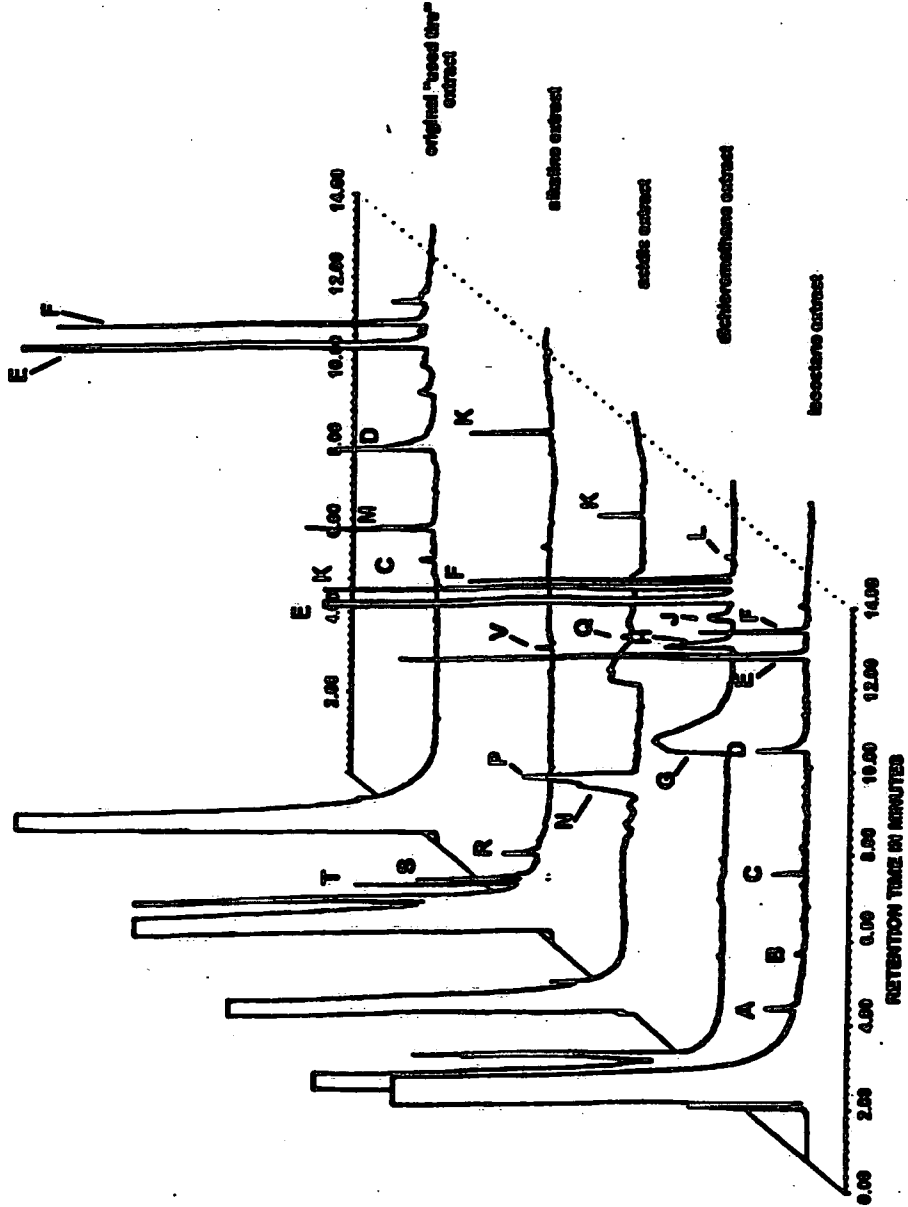
Figure 3B Comparative FTIR chromatograms of 0.5% salinity leachates



**Most Probable Identification**

- A 2° or 3° alcohol
- C Unsaturated ketone
- D Morpholinotio benzothiazole
- E Nitro 1
- F Nitro 2
- H Nitro 1A
- Ia - Ig paraffins
- J Nitro -OH
- K phthalate (ethylhexyl)
- L Nitro 3
- M Alkyl ether der.
- N Carboxylic acid
- P Carboxylic acid (2,2-dimethyl der.)
- Q Lactone
- R 2-chlorocyclohexanol
- S Toluene
- T 1° amine
- V 1° alcohol
- W Cyclohexene oxide

Figure 3C Comparative FTIR chromatograms of 2.5% salinity leachates



**Most Probable Identification**

- A 2° or 3° alcohol
- B Benzothiazole
- C Unsaturated ketone
- D Morpholinothio benzothiazole
- E Nitro 1
- F Nitro 2
- G triazine der. ?
- H Nitro 1A
- J Nitro -OH
- K phthalate (ethylhexyl)
- L Nitro 3
- M Alkyl ether der.
- N Carboxylic acid
- P Carboxylic acid (2,2-dimethyl der.)
- Q Lactone
- R 2-chlorocyclohexanol
- S Toluene
- T 1° amine
- V 1° alcohol

Figure 4A Total Ion Chromatogram and Mass Spectra of Isooctane extract

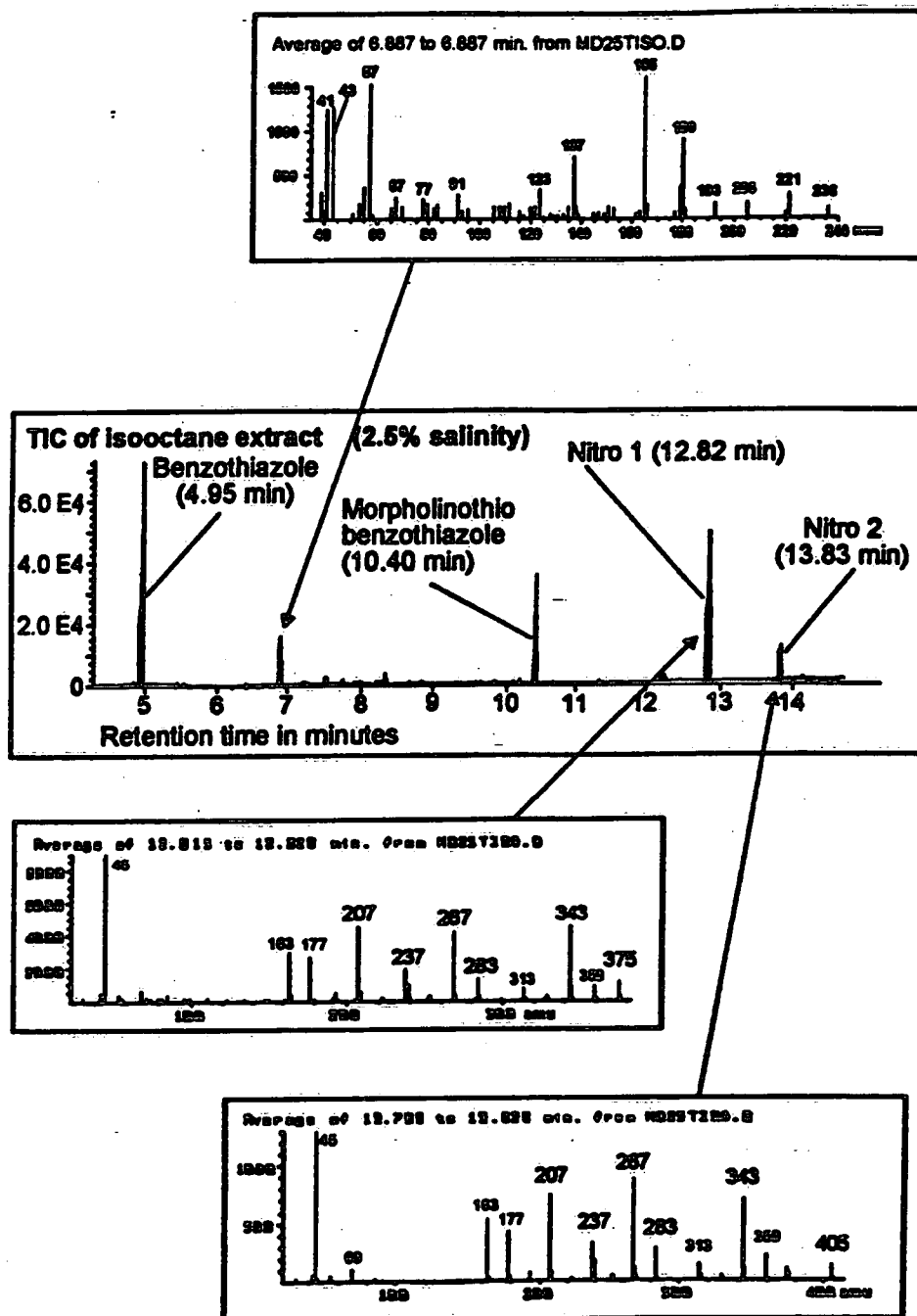




Figure 4B Total Ion Chromatogram and Mass Spectra of Dichloromethane extract

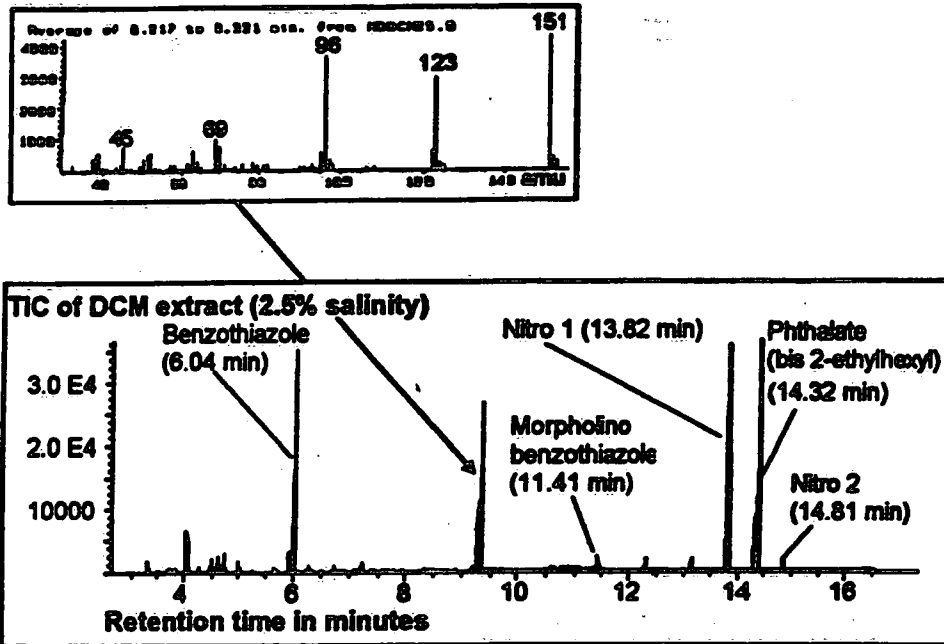


Figure 4C Total Ion Chromatogram and Mass Spectra of Acidic DCM extract

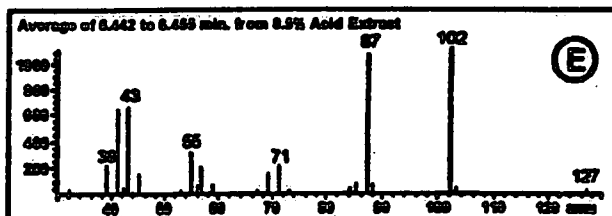
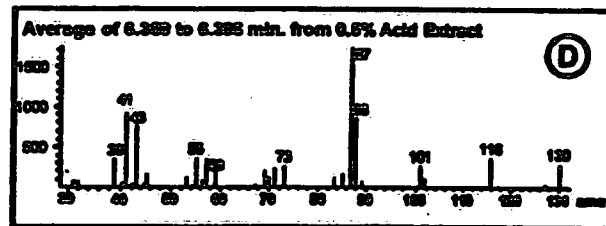
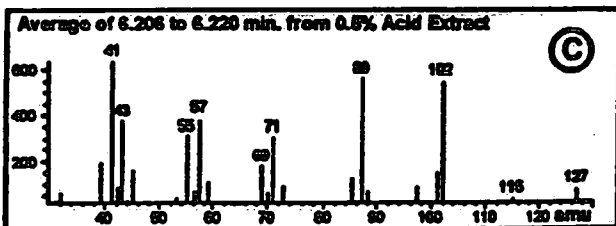
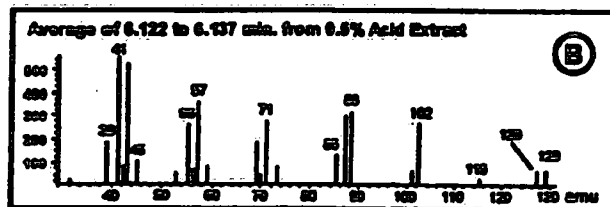
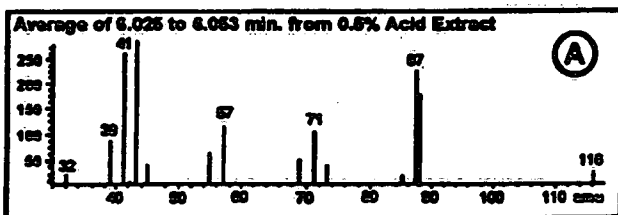
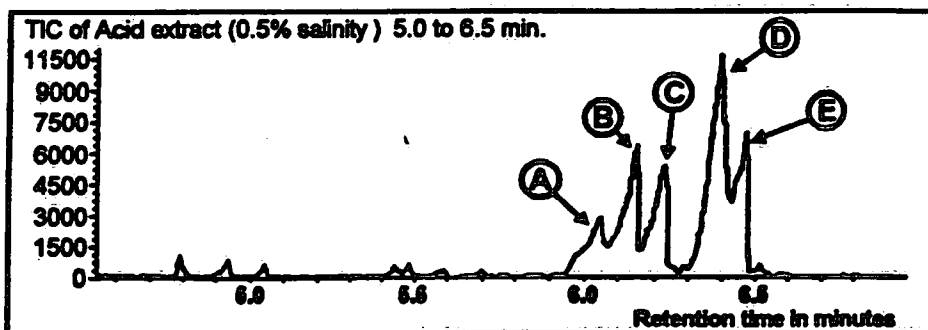
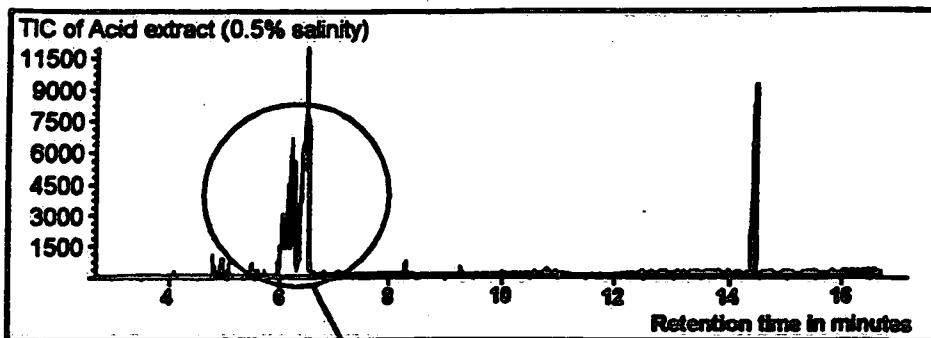
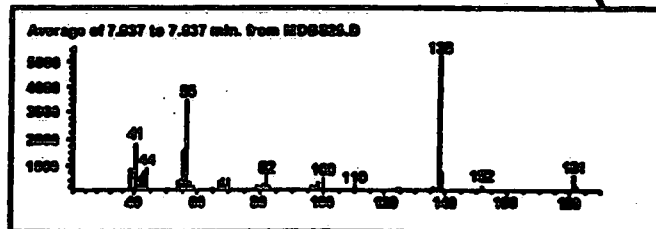
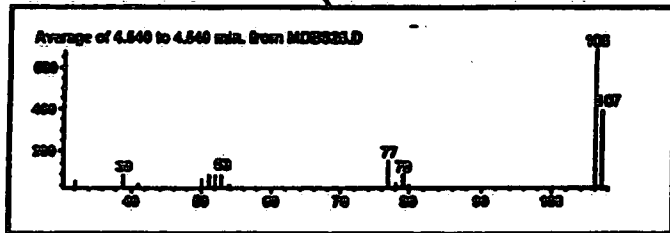
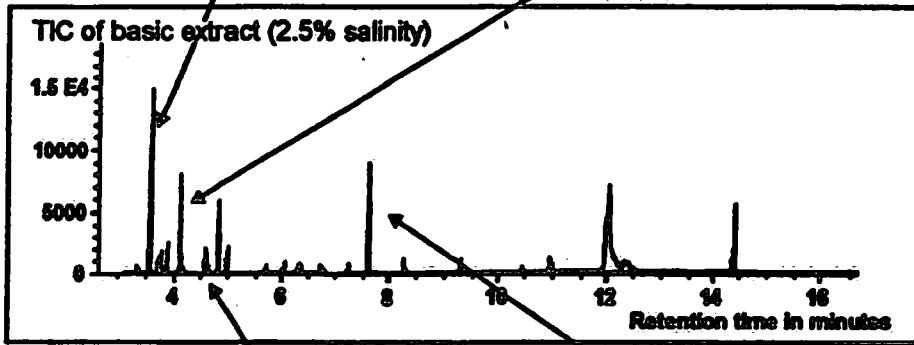
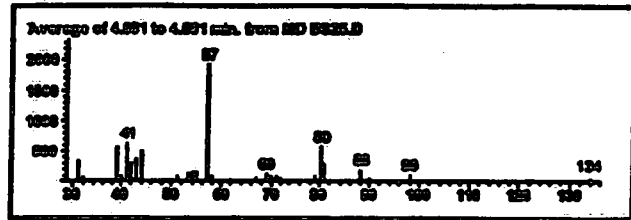
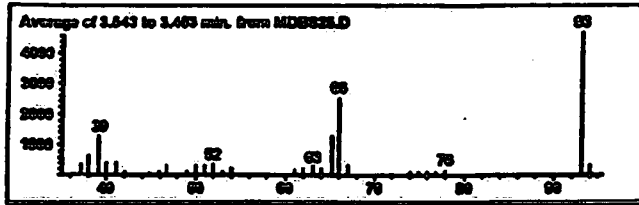


Figure 4D Total Ion Chromatogram and Mass Spectra of Alkaline DCM extract



**Figure 5. Thin Layer Chromatograms of shredded tire extracts [Toluene : Acetone (7:3) vol/vol]**

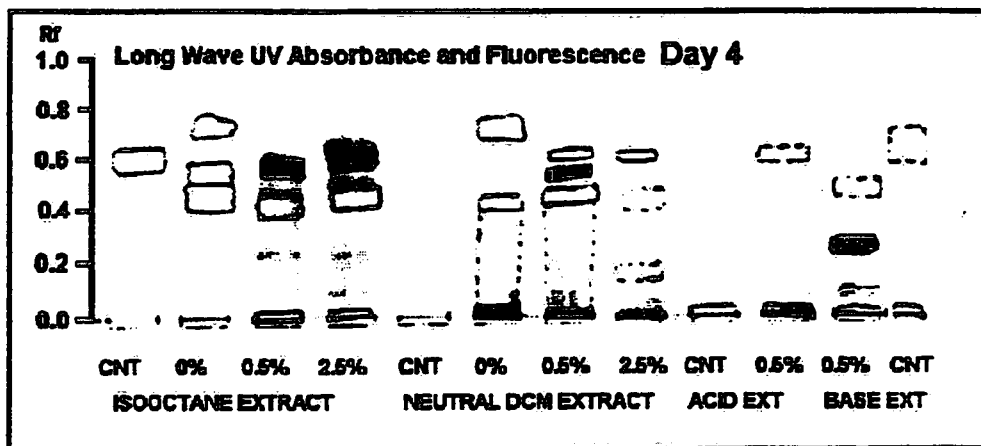
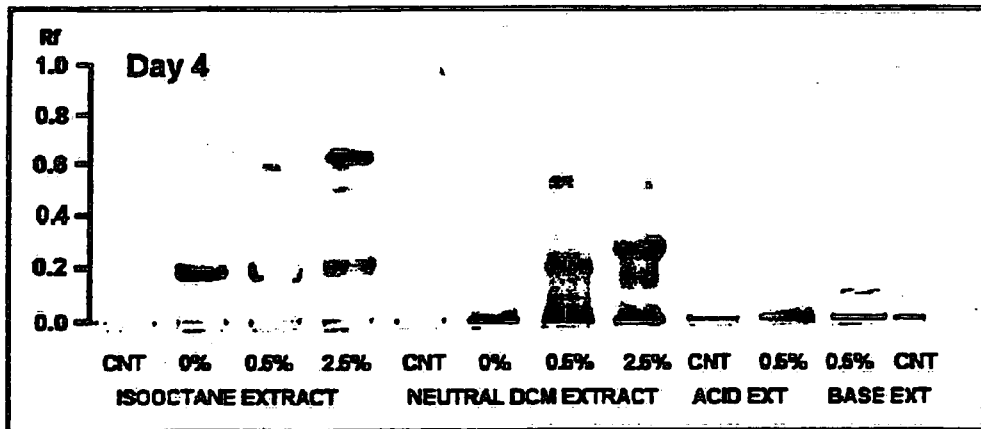
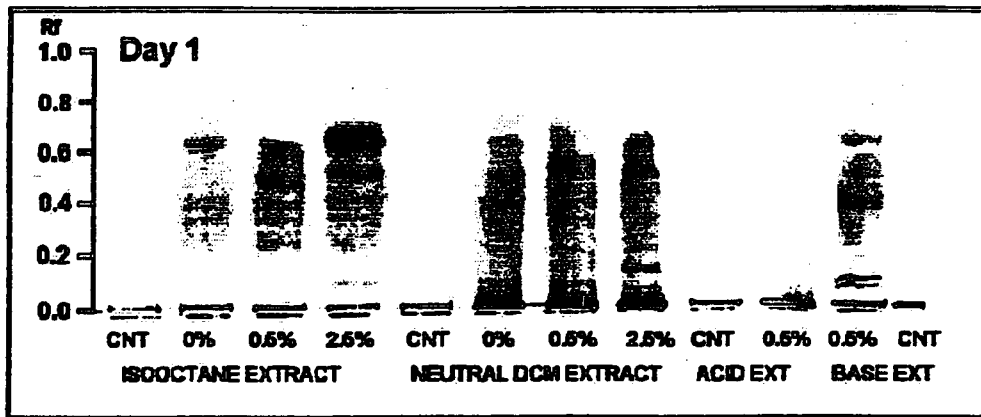


TABLE I: Sample Characteristics and Sample Processing Data

Sample Designation	Function	Salinity (% w/v) (ppth)*	Sample Volume (mL)	pH	Comments	Final Volumes of Concentrates (mL) and [concentration factors]				
						UV/VIS	GC/MSD	GC/FTIR	TLC	Microtox
"25" Control	Process Blank	2.5 (25)	2490	7	Some flocculent material, yellow colour associated with precipitate, not aqueous phase	4.0 [623]	1.25 [1992]	1.25 [1992]	.100 [-25,000]	4.0 [623]
"0" Leachate	Leachate	0.0 (0.0)	2290	6	-	4.0 [573]	1.15 [1991]	1.15 [1991]	.100 [-23,000]	4.0 [573]
"5" Leachate	Leachate	0.5 (5)	3290	6	Some fine rubber bits	4.0 [823]	1.65 [1994]	1.65 [1994]	.100 [-33,000]	4.0 [823]
"25" Leachate	Leachate	2.5 (25)	3220	7	-	4.0 [805]	1.61 [2000]	1.61 [2000]	.100 [-32,000]	4.0 [805]

\* The designation "ppth" (parts per thousand) is used in this report to distinguish from the designation "ppt" (parts per trillion - ppt) which is commonly used in reporting results of environmental analyses.

**Table II**  
**Normalized IR GSP peak heights (iso-octane extract)**

Compound	0 ppth	5 ppth	25 ppth	Control
Rt 2.51	47	32	13	nd
Benzothiazole	nd	nd	4	nd
Rt 5.56	18	19	12	nd
Morpholino Bnzt.	24	22	17	nd
Nitro 1	55	89	133	nd
Nitro 2	16	19	35	nd

**Normalised IR GSP peak heights (DCM extract) Different oven temp. profile**

Compound	0 ppth	5 ppth	25 ppth	Control
Rt 1.79	45	15	nd	nd
Rt 2.21	42	9	nd	nd
Rt 2.29	19	7	nd	nd
Rt 3.49	12	nd	nd	nd
Rt 10.50 nitro-?		17	28	nd
Rt 11.17 nitro-0		37	28	nd
Rt 11.39 nitro-OH	42	nd	nd	nd
Rt 11.69 nitro-1		200	250 <sup>a</sup>	nd
Rt 12.00 phthalate	134	150	135	250
Rt 12.17 nitro-2		51	118	no

**Normalised [2000 fold conc.] IR GSP peak heights (Acid extract)**

Compound	0 ppth	5 ppth	25 ppth	Control
Rt 5.39	9	14	20	nd
Rt 5.64	20	30	38	nd
Phthalate	37	55	15	33

**Normalised [2000 fold conc.] IR GSP peak heights (DCM extract)**

Compound	0 ppth	5 ppth	25 ppth	Control
Rt 1.05	5	63	65	250
Rt 6.87	nd	6	5	nd
Phthalate	6	22	25	250

nd not detected

<sup>a</sup> represents linear limit of IR detector response

**TABLE III: Extractable Residue Weights of Solvent Fractions  
mg (+/- 0.5 mg)**

Solvent Fraction	isooctane	DCM	acidic DCM	alkaline DCM	Total
<u>Salinity</u>					
0 ppth	3.1	7.7	3.8	0.4	14.9
5 ppth	5.4	11.6	2.9	0.0	19.9
25 ppth	3.9	12.2	3.2	0.6	19.9
Control (25 ppth)	0.7	0.6	1.1	0.3	2.7

Total calculated extractable residue weight  
for "0" ppth shredded tire sample: 48.9 mg

Total calculated extractable residue weight  
for whole-tire sample ("new" tire, (14)): 66 mg

Estimated total leachable surface area of  
tire material in "0" ppth leaching test: 924<sup>2</sup> cm

Estimated total leachable surface area  
of tire material in whole-tire test (14): 13,500<sup>2</sup> cm

**Table IV ICP Metals Analysis for Whole and Shredded Tire Samples (Results in mg/L)**

Metals	Shredded Tire samples				Whole Tire samples		
	25 ppt control	0 ppt leachate	5 ppt leachate	25 ppt leachate	Control	Used	New
Al	0.11	0.06	0.05	0.07	0.02	0.01	0.03
Ba	0.129	0.0841	0.086	0.11	0.105	0.108	0.119
Be	0.0002	<0.0002	<0.0002	0.0002	<0.0002	<0.0002	<0.0002
Ca	193	4.7	49.8	181	32	42.3	34
Cd	0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001
Co	0.002	0.005	0.005	0.004	<0.001	<0.001	<0.001
Cr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cu	0.007	0.007	0.004	0.011	<0.001	<0.001	<0.001
Fe	0.027	1.36	0.555	0.003	<0.001	<0.001	<0.001
K	366	3.8	65.6	318	1.6	1.1	1.1
Li	156	0.123	3.24	133	0.043	0.006	0.008
Mg	1516	12	258	1364	33.5	29.4	29.5
Mn	0.0037	0.112	0.172	0.29	0.0012	<0.0005	<0.0005
Mo	0.002	<0.001	<0.001	0.001	0.001	0.001	0.001
Na	7340	73.3	1242	6580	25.1	6.6	7.3
Ni	0.002	0.006	0.005	0.006	<0.002	<0.002	<0.002
Pb	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Sr	8.96	0.0762	1.56	7.82	0.198	0.187	0.181
V	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Zn	0.031	3.38	2.21	0.364	0.006	0.005	0.006



**TABLE V: Microtox Toxicities of Solvent Fractions of the "0" ppth Leachate and of Whole-tire Leachate**

"0" ppth leachate extract fraction	Microtox Toxicity ( $\Gamma$ , (16)) *
Isooctane	3.78
DCM, neutral pH	10.64
DCM, pH 2	50.80
DCM, pH 12	0.12
Whole-tire leachate extract (new" tire, (16))	8.1
Whole-tire leachate extract ("used" tire, (16))	4.2
Isooctane blank	0.05
DCM blank	0.07

\* Normalized to a 1000-fold concentration factor

The gamma ( $\Gamma$ ) value is used in the Microtox test in cases for which the molecular weight of the test substance is unknown.

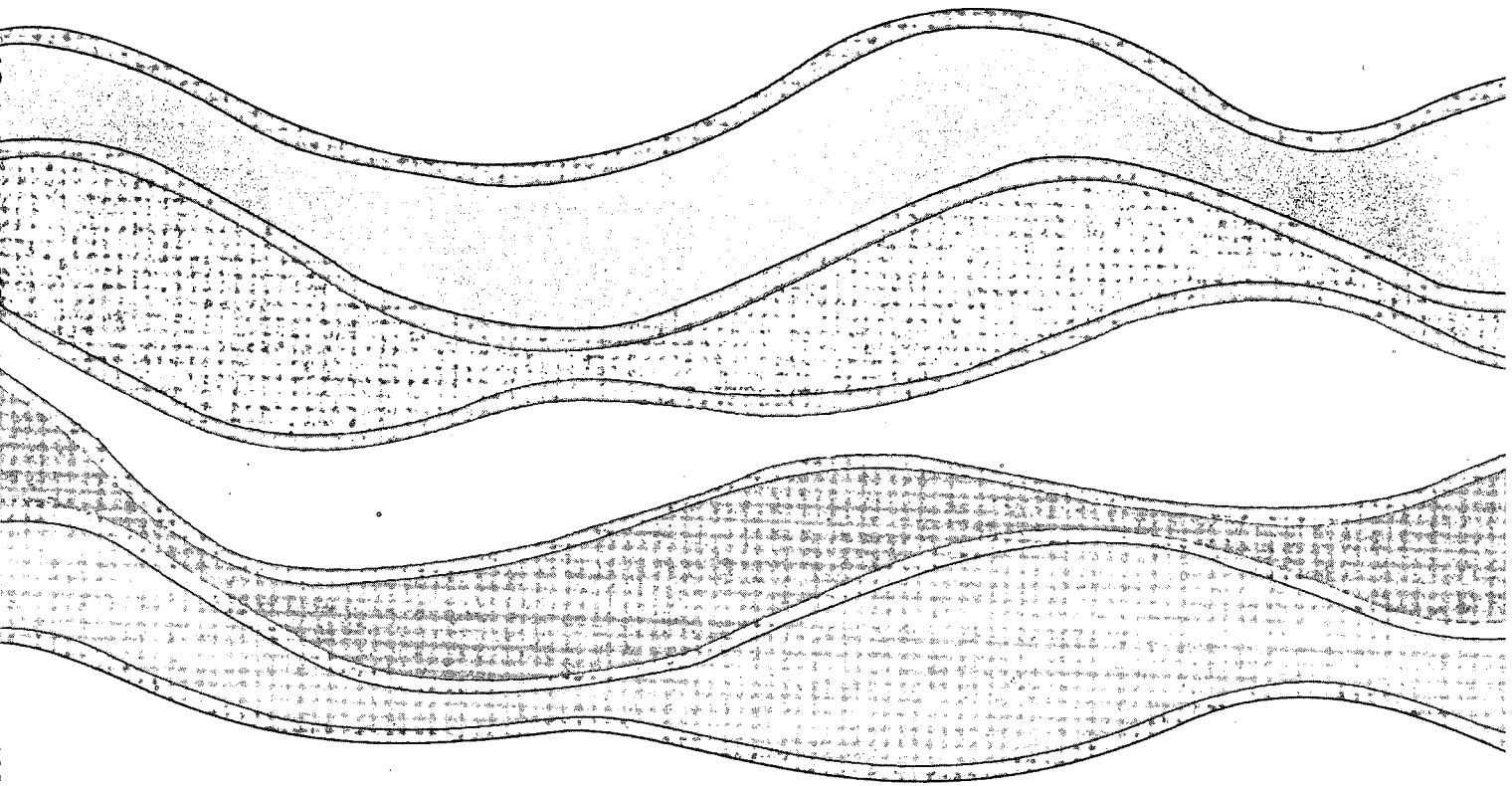


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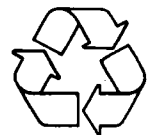
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