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# RESEARCH PROJECT SUMMARY

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# ISOLATION AND IDENTIFICATION OF SYNTHETIC ORGANIC CHEMICALS IN DRINKING WATER

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## ABSTRACT

Chemical contaminants in drinking water are often present at low concentrations. To identify and characterize these contaminants using biological assays and/or advanced chemical analytical techniques it is necessary to extract and concentrate these contaminants. Large volumes of drinking water, typically 100 to 500 liters, must be extracted with organic solvents, and the extract concentrated to obtain sufficient levels of contaminants for analysis.

A prototype high volume 10 liter per hour (L/hr) Continuous Liquid-Liquid Extractor (CLLE) was developed and implemented in this project. The extractor is portable and completely self contained, making on-line continuous monitoring at water treatment purveyor sites possible.

In a coordinated multidisciplinary effort between NJDEPE Division of Science and Research, Drexel University, Coriell Institute for Medical Research, and Rutgers University, a significant scientific advancement in sample extraction methodology and understanding of the chemistry and mutagenic activity of synthetic organic chemical contaminants in drinking water has been accomplished. The results of this research give insight into the method selectivity and compound identification capabilities at the part per trillion level in drinking water.

## ABBREVIATIONS

| | |
|---|---|
| **CLLE:** | Continuous Liquid-Liquid Extractor |
| **DCM:** | Dichloromethane or Methylene Chloride, (EM Science) |
| **DOC:** | Dissolved Organic Carbon ($\mu$g/L as C) |
| **EOM:** | Extractable Organic Matter (non-volatile residue) |
| **GAC:** | Granular activated carbon |
| **GC/MS:** | Gas Chromatography/Mass Spectrometry |
| **Milli-Q Water:** | Ultra-pure deionized Water, (Millipore Corp.) |
| **PBLC/MS:** | Particle Beam Liquid Chromatography/Mass Spectrometry |
| **QA/QC:** | Quality Assurance/Quality Control |
| **S9:** | Enzyme-induced liver homogenate from laboratory rats (Wistar strain) plus co-factors |
| **TA98:** | Genetically modified *Salmonella typhimurium* bacterium used in the Ames mutagenicity assay |
| **TA100:** | Genetically modified *Salmonella typhimurium* bacterium used in the Ames mutagenicity assay |
| **USEPA:** | United States Environmental Protection Agency |
| **XAD Resins:** | Hydrophobic (styrene divinylbenzene) solid adsorbent, (Rohm & Haas Corp.) |

**UNITS:** L = liter, mL = milliliter ($10^{-3}$ liter), $\mu$g = microgram ($10^{-6}$ gram), $\mu$m = micrometer or micron ($10^{-6}$ meter), part per trillion = nanogram per liter (ng/L) = ($10^{-9}$ grams per liter)

## INTRODUCTION

This report summarizes a continuing project initiated in 1988, which studied the fundamental problem of identifying and characterizing chemical contaminants in drinking water. The resources of three institutions (Dr. Suffet of Drexel University-high volume specialized sampling, Dr. Joseph Rosen and Robert Rosen of Rutgers University-sophisticated instrumental analysis, and Dr. Thomas Atherholt of the Coriell Institute for Medical Research-Ames mutagenicity analysis), were employed to isolate, chemically identify and determine the mutagenic activity of trace organic compounds in potable water.

## OBJECTIVES

The objectives of this research were:

- Design and optimize a high volume continuous liquid-liquid extractor (CLLE) capable of isolating trace organic compounds from the raw (untreated influent) and finished (treated) drinking water in a reproducible manner.
- Develop a quality assurance/quality control (QA/QC) program capable of validating the CLLE isolation method,
- Field test the high volume 10 L/hr CLLE at several drinking water purveyors in New Jersey by extracting 500 liters of raw and finished drinking water at each site and analyzing the concentrated extracts using advanced instrumental analysis and the Ames mutagenicity bioassay [1].

## 10 L/hr CLLE DESCRIPTION

The Continuous Liquid Liquid Extraction (CLLE) isolation methodology developed in this research is based upon the promulgated USEPA Method 625 in which organic compounds are extracted using dichloromethane (DCM) solvent and concentrated by solvent evaporation techniques [2].

In preliminary field research, a 2 L/hr CLLE prototype was used. A 2 ml CLLE extract volume of one hundred liter quantities of finished drinking water (50,000-fold enrichment) proved adequate for the detection of acid/base neutral compounds by GC/MS and measurable Ames Bioassay response. To detect organic compounds by other instrumental methods, such as Particle Beam LC/MS, and MS/MS a further increase in concentration above the 50,000 fold level is required. Therefore, the extraction of large volumes of drinking water is required for advanced instrumental analysis and bioassay determinations. In addition, an adequate flow rate through the extractor must be obtained to isolate sufficient quantities of organic matter to address daily temporal and spacial variability. The high volume 10 L/hr cocurrent continuous liquid-liquid extractor (CLLE) employed in this research is conceptually based on a 2 L/hr CLLE design by Wu and Suffet (1977) [3].

The 10 L/hr CLLE, illustrated in Figure 1, is constructed of three components. The first component is the solvent/water injection system. The CLLE system is connected on-line at the water treatment facility to a ceramic reciprocating pump at a flow rate of 10 L/hr. The DCM extracting solvent is pumped through a second, identical metering pump at a rate of 1 L/hr from a reservoir which collects distilled solvent from the recovery condenser. The solvent and water are combined into a cocurrent stream through a Teflon T-union.

The second components are two parallel mixing coils which allow sufficient contact time for the extraction to occur. Extraction of the water contaminants occurs due to DCM/water partition coefficients which are chemical specific. Each coil consists of a 32 ft, 1/8 inch I.D. Teflon line coiled around a 4 inch diameter support pipe.

The final glass component consists of a phase separation chamber, delivery loop, and solvent recovery system. The phase separation chamber allows extracted water to exit the CLLE to waste by gravity decanting. The heavier than water solvent is conducted through a delivery loop to the bottom of a distillation stack. The solvent is reclaimed by a recovery condenser while the trace organic compounds are retained in the stack. Final concentration of the extract, following extraction, is achieved by controlled temperature evaporation of the DCM. The 5 mL DCM extracts obtained from field studies

represent a 100,000 fold enrichment of the chemicals in 500 liters of drinking water. The CLLE is portable and completely self contained, making on-line, continuous monitoring at water treatment purveyor sites possible.

Intrinsic and extrinsic water quality parameters will affect the collection of extractables. Therefore, pH, DOC, conductivity, residual chlorine determinations, temperature of influent water, analysis times, and extractable organic matter (EOM), were monitored during the CLLE extraction.

## QUALITY CONTROL

To develop and validate an extraction and concentration methodology, meticulous care was exercised at every step in the analysis. A rigorous QC protocol was developed for the CLLE methodology.

The program involved the use of;

- **Solvent blanks** to check the acceptability/reactivity of the solvent,
- **45 liter Recovery spikes** (addition and recovery of pure compounds) to judge the performance of the extractor design and determine any degradation of the test compounds,
- **100 liter Milli-Q water Blanks** to simulate the effect of water saturated solvent and recovered preservative levels[1],
- **Chlorinated, Chloraminated, and pH Modified 100 liter Milli-Q water blanks** to determine acceptability of pH modifiers and dechlorination agents, and
- **Equipment blanks** to certify CLLE cleanliness.

## 10 L/HR CLLE PERFORMANCE CRITERIA

### A. COMPARISON WITH USEPA METHOD 625

The CLLE exceeds the performance criteria for method precision and accuracy for recovery of several test analytes specified in the 625 Method. Five of the thirteen compounds contained in the recovery test mixture were compounds listed in Method 625; isophorone, naphthalene, 1,4 dichlorobenzene, 2,4 dichlorophenol, and anthracene. A comparison in the range of recovery efficiencies between the CLLE and the USEPA Method 625 is shown in Table 1. The CLLE recovery data were determined from four recovery experiments, each using two 10 L/hr CLLE's. The Method 625 recovery range was calculated from the literature reported mean recovery values and standard deviations for the compounds listed in the method. In most instances, the accuracy range for the CLLE fell within the performance criteria for the standard method. The notable exception was 2,4 Dichlorophenol which has a high range CLLE value of 107% versus 101% for Method 625. In all cases the CLLE has superior accuracy, demonstrated by higher low-range values, and better precision, indicated by a tighter range of recovery values.

### B. FIELD PERFORMANCE RESULTS

Figure 2 illustrates the results of both raw and finished drinking water at three sites in New Jersey. The raw and finished drinking water extractions were determined on the same day using two 10 L/hr CLLE's at each site. The detention time of the facility determined the start time of the finished drinking water CLLE. The raw and finished CLLE extracts were chemically analyzed and the data was compared to investigate the organic removal efficiency of the water treatment process. Similar quantities of DOC and of EOM were determined at each facility. The sum of the total identified chemical mass using GC/MS and PBLC/MS is also shown in Figure 2. The data shows the utility of using PBLC/MS, in addition to GC/MS, since the two

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[1]DCM contains a preservative, (in this case cyclohexene), which inhibits the formation of phosgene gas, a degradation product of methylene chloride. Solvent preservatives undergo the same concentration conditions as the trace organic compounds. The preservatives react with residual chlorine in finished drinking water to form a variety of products. The QC samples simulated actual field conditions and reactivity of these preservatives [4]

methods detect different types of compounds. The data also shows the total mass which current instrumental techniques can identify. On average 5 % of the total EOM was characterized by GC/MS and PBLC/MS. This identified fraction is only 0.1 % of the total DOC present in drinking water.

Temporal variability in the organic matrix has been identified by sequential time based composite CLLE extraction and analysis. In this experiment, two different CLLE extractors were run during different time periods at the same water treatment plant. The resulting chromatographic data indicated different compounds with different concentrations in the same source water.

### C. AMES MUTAGENICITY RESULTS

Figure 3 shows the results of the Ames bioassay of extracts. This assay uses genetically altered Salmonella bacteria to detect chemicals capable of mutating DNA. Such compounds have the potential to cause cancer or birth defects. Mutagenic activity is expressed as mutated bacterial colonies (revertants) per liter of drinking water. The results of analysis of extracts of 500 liter samples from twelve drinking water purveyors in New Jersey indicated the presence of little or no mutagenic compounds in untreated (raw) waters, with assay values ranging from 0 to 12 mutated bacterial colonies (revertants) per liter. The mutagenic activity in 500 liter finished drinking water extract mutagenic responses ranged from 40 to 645 revertants per liter . Maximum mutagenic activity was observed in the absence of rat liver homogenate metabolic activation components (S9). Mutagenic activity levels were positively correlated with nonvolatile residue levels.

## CONCLUSIONS

The organic compounds identified in this research were found at concentrations approximately two orders of magnitude below the method detection level for the promulgated USEPA Method 625, and therefore have **not been previously identified** in finished drinking water. The use of advanced instrumental techniques such as Particle Beam LC/MS have **doubled** the identifiable mass of organic contaminants in extracts of drinking water [5].

Most of the compounds responsible for the mutagenic activity appear following the chlorine disinfection process. Identified compounds which were present in both the raw and finished drinking water extracts can be eliminated as possible contributors to the mutagenic activity detected in drinking water. The contribution of the remainder of the compounds identified and the humic substances which constitute the majority of the EOM has yet to be determined.

The results of the temporal variability study indicate that periodic batch sampling methodologies currently employed for the analysis of compounds in finished drinking water may not be representative of actual environmental exposures to organic compounds present in drinking water. Since the levels of many of the compounds identified in this research are in the part per trillion range, conventional instrumental analysis cannot currently detect these chemicals. By evaluating both the raw and finished drinking water at various purveyors, a measure of the effectiveness of the treatment process has been obtained. Some synthetic organic compounds pass through the facilities unaffected by the unit operations of filtration, chlorination, and GAC. The CLLE isolation methodology, coupled with advanced instrumental analysis can be used to evaluate organic compound removal efficiencies in each treatment operation at drinking water purveyor sites.

## RECOMMENDATIONS

The list of semiquantitative concentrations of compounds identified in this project will be submitted to the Lists and Levels Subcommittee of the New Jersey Drinking Water Quality Institute for evaluation and recommended course of action.

Future research should address the use of solid adsorbents (eg. XAD resins) which are have the capacity to extract polar organic compounds not isolated by DCM.

In addition to the advanced analytical instrumentation used in this research, tandem MS/MS can be evaluated as another identification tool. MS/MS may detect biomolecules and other high molecular weight organic compounds not previously identified.

The Ames mutagenicity bioassay detected

mutagens in chlorinated drinking water samples. Many of these mutagens have not been chemically identified. Methods need to be developed to identify the components responsible for the mutagenic activity.

## REFERENCES


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## FUNDING SOURCE AND AVAILABILITY OF FINAL REPORT


This research effort was funded through the New Jersey A-280 Drinking Water Program. The Final Report on this project is available from the Division of Science and Research (609 292-9692). To obtain more detailed information regarding this research, two Ph.D. dissertations are maintained on file and are available for reference and review. General information about the environmental research program at the Division of Science and Research is also available (609 984-6071).

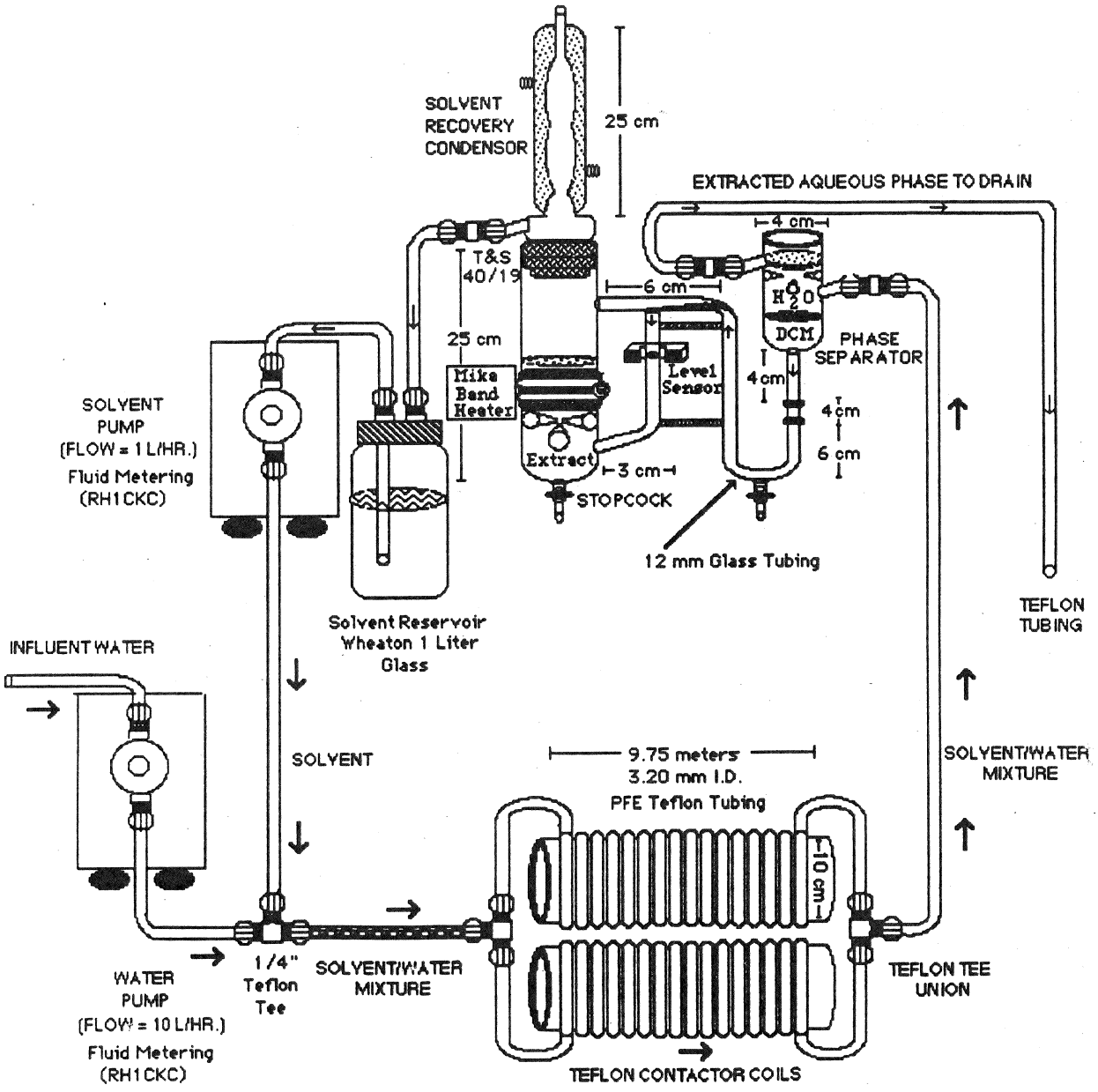

## PROJECT PARTICIPANTS AND AFFILIATIONS


Dr. I.H. Suffet is currently affiliated with the Environmental Science and Engineering Program, Dept. of Environmental Health Sciences at UCLA School of Public Health. Drs. Joseph Rosen and Robert Rosen are Faculty members of Rutgers University's Center for Advanced Food Technology at the Cook College Campus. Dr. Thomas Atherholt is presently with the Division of Science and Research, NJDEPE.


## ACKNOWLEDGMENTS

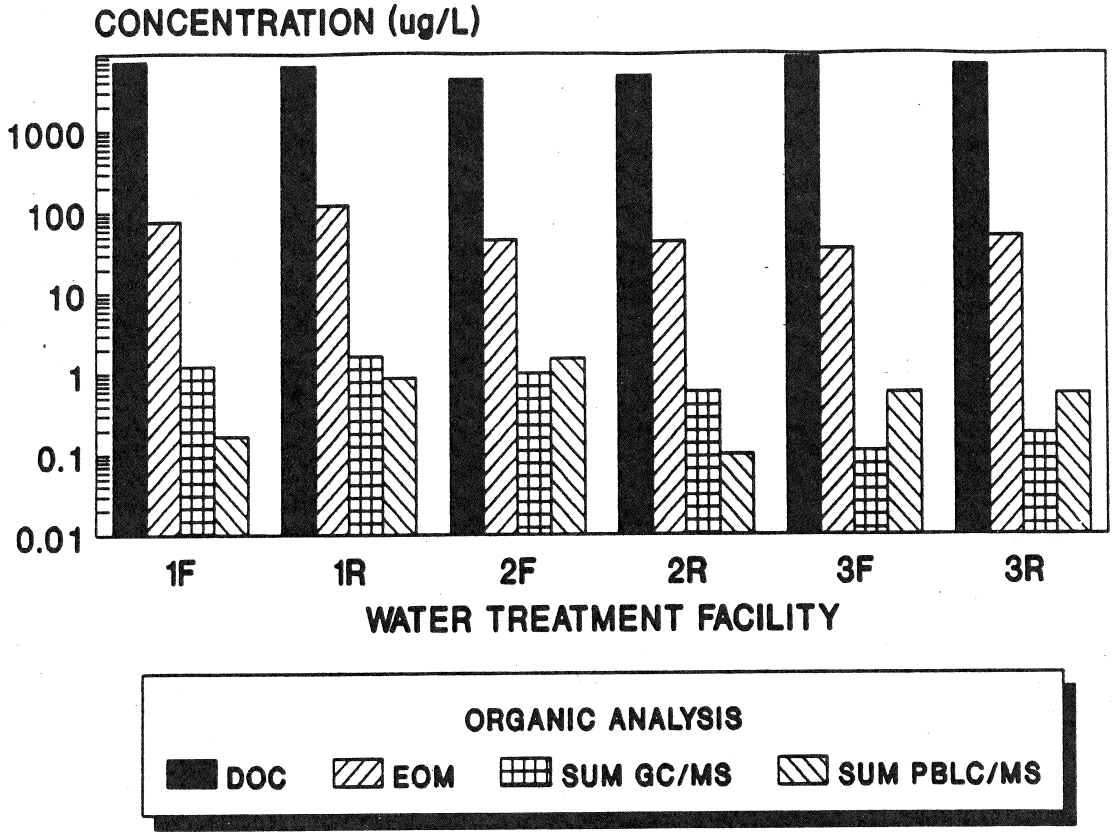

The authors of this research effort would like to thank Project Managers Drs. Judy Louis and Chuck Hertz for the access and selection of the various water treatment purveyors, and Dr. Linda Clark, for identification of the organic compounds by GC/MS and PBLC/MS.

# Figure 1: 10 L/hr CLLE



# Figure 2:

## CONCENTRATION OF ORGANIC MATERIAL IN RAW AND FINISHED DRINKING WATER

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| **DOC** | DISSOLVED ORGANIC CARBON<br>(μg/L as C)<br>[Dohrman total carbon analyzer] |
| **EOM** | EXTRACTABLE ORGANIC MATTER<br>Gravimetric residue from DCM extracts (Cahn Electrobalance) |
| **F** | FINISHED (TREATED) DRINKING WATER |
| **R** | RAW (UNTREATED INFLUENT) WATER |
| **Sum GC/MS** | The sum of the concentrations of GC/MS identified compounds |
| **Sum PBLC/MS** | The sum of the concentrations of PBLC/MS identified compounds |

# Table 1:

## USEPA METHOD 625 VS. CLLE RECOVERY
### *PRECISION AND ACCURACY COMPARISON*

| RECOVERY COMPOUND | 625 RANGE (%) | CLLE RANGE (%) |
|---|---|---|
| ANTHRACENE | 54 - 98 | 57 - 89 |
| ISOPHORONE | 42 - 108 | 81 - 93 |
| 2,4-DICHLOROPHENOL | 59 - 101 | 81 - 107 |
| NAPHTHALENE | 4[illegible] - 110 | 45 - 97 |
| 1,4-DICHLOROBENZENE | 2[illegible] - 98 | 46 - 82 |

# Figure 3:

AMES ASSAY MUTAGENIC ACTIVITY FOR
N.J. DRINKING WATER PURVEYORS