United States
Environmental Protection
Agency

Research and Development

Environmental Monitoring
Systems Laboratory
Las Vegas NV 89193-3478

EPA/540/SR-93/518 September 1993

EPA

Project Summary

Superfund Innovative Technology Evaluation (SITE) Program Evaluation Report For Antox BTX Water Screen (BTX Immunoassay)

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The results of a demonstration of a portable immunoassay for the detection of benzene, toluene, and xylene(s) (BTX) are described in this report. The BTX immunoassay was developed by Antox, Inc., (South Portland, ME) and is intended as a screening technology. Seventy-nine field samples were obtained from monitoring wells at several sites with gasoline-contaminated ground water. Sample splits were analyzed on-site by the BTX immunoassay and in the laboratory by gas chromatography (GC) using EPA Method 8020.

The BTX immunoassay was rapid and simple to use. It performed well in identifying high-level contamination and gasoline-contaminated samples having BTX concentrations greater than 100 ppb. It did not fully meet the claims of the developer of identifying contamination levels down to 25 ppb BTX. Two field samples determined by GC to have between 25 and 100 ppb BTX failed to be classified correctly by the immunoassay. Results from quality assurance samples with BTX concentrations of 2.5, 25, and 100 ppb also showed that false negative results would be expected at higher than a 5 percent rate when BTX contamination levels were between 25 and 100 ppb. However, for samples with higher BTX levels, the immunoassay gave excellent results. Two field samples yielded false positive results compared to GC values, but these samples showed signs of low-level gasoline contamination. This technology could provide cost-effective screening or monitoring functions at sites with ground water contaminated by gasoline or other fuels. It could also be used in a laboratory setting to select samples for further analysis.

This Project Summary was developed by EPA's Environmental Monitoring Systems Laboratory, Las Vegas, NV, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The performance of an immunoassay field kit technique was evaluated during a U.S. Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) demonstration. This semi-quantitative method was developed by Antox Inc. (South Portland, ME), to detect benzene, toluene, and xylene (BTX) contaminant levels in environmental water samples. The evaluation compared results from BTX immunoassays performed in the field to quantitative results obtained by laboratory-based gas chromatography (GC) using EPA Method 8020.

Technology Description

The BTX immunoassay is a competitive enzyme-linked immunosorbent assay (ELISA), which uses an antibody-coated polystyrene cuvette as the solid phase. A hapten-enzyme conjugate mimics the free analyte (BTX compounds) and competes with aromatic compounds in the sample for binding sites on the immobilized antibody. The test is performed by placing

reference and test solutions in separate coated cuvettes in which competition for binding to the antibody occurs. After a 10-minute incubation step, substrate and chromogen are added, and a colored enzymatic reaction product is formed. The optical density (OD) of the reference and sample cuvettes is determined using a portable colorimeter, and the ratio of sample to reference OD values is determined. The color intensity is inversely proportional to the BTX hydrocarbon level in the test sample. Low sample-to-reference ratios are associated with high BTX concentrations and high ratios with low BTX concentrations. The developer had selected a ratio of 0.85 as the decision level, with all samples having a ratio less than 0.85 being labeled as having 25 ppb BTX or greater.

The laboratory confirmatory method was gas chromatography following EPA Method 8020 using the purge-and-trap sample preparation technique in EPA Method 5030.

Demonstration Design

Seventy-nine field samples were obtained from four separate monitoring wellfields in the Las Vegas valley during the first quarter of 1992. Three of these sites represented gasoline sources and the fourth site had wells that might also be contaminated with other hydrocarbon mixtures, such as jet fuel or diesel fuel. Each sample well was purged of three well-volumes prior to sampling to remove stagnant water from the well and surrounding aquifer. Sample splits were placed in clear 40-mL volatile organic analysis (VOA) vials with Teflon septums. The field immunoassay sample split was run in duplicate on-site and the other sample splits were maintained at 4°C until analysis. In addition to sample blanks, a variety of quality assurance samples were prepared having concentrations from 2.5 to 100 ppb BTX. These samples were used to check for sample stability, transportation and storage problems, and to verify the performance characteristics of the GC method.

Results and Discussion

Of the 79 field samples, 36 were determined to be above 25 ppb BTX by GC analysis while 43 samples had concentrations below the 25 ppb criteria of the immunoassay. Two (six percent) of the positive samples gave false negative results on both replicate analyses. One of these samples had 35.5 ppb and the other had 74.8 ppb BTX. Two (five percent) of the negative samples gave false positive results on both replicate analyses. However, each of these samples was associated with minor levels of gasoline contamination.

A plot of sample to reference OD ratios vs. BTX concentration as determined from GC analysis revealed the expected sigmoidal curve shape. However, there was significant variability in the S/R ratio for a given concentration level. In the region of 25 to 100 ppb BTX, this variability hindered the immunoassay from performing up to the developer's expectations. Evaluation of quality assurance samples having concentrations from 2.5 to 100 ppb BTX also showed that a greater than five percent chance of false negatives would be expected up to approximately 100 ppb BTX. For higher level samples, the immunoassay worked quite well.

Conclusions and Recommendations

The BTX immunoassay performed well for most samples evaluated in this study. The immunoassay was portable, easy to use, inexpensive, and provided a rapid estimate of BTX concentration that would prove useful in certain applications. For instance, this method could prove useful in mapping the distribution of contaminants at a site or for monitoring changes in contaminant levels over time. Samples with BTX concentrations above 100 ppb were accurately identified. Only five percent of the samples were misclassified based on replicate determinations. The false negative and quality assurance results suggest that the current formulation of the BTX immunoassay is not robust enough to accurately deal with samples in the range of 25 to 100 ppb BTX (as the manufacturer claims). If one wants to be very accurate in this range, a more sensitive format should be developed. An additional area for development that was identified by this demonstration was the need for a stable low-level performance standard or a simple procedure for generating these types of standards.

The information in this document has been funded wholly or in part by the U.S. Environmental Protection Agency under Contract Number 68-CO-0049 to Lockheed Engineering & Sciences Company. It has been subjected to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

☆U.S. GOVERNMENT PRINTING OFFICE: 1993 - 750-071/80078

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The complete report, entitled "Superfund Innovative Technology Evaluation (SITE) Program Evaluation Report For Antox BTX Water Screen (BTX immunoassay)," (Order No. PB93-218337; Cost: $27.00; subject to change) will be available only from:

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