TA-19

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Date: December 9, 2005
Refer to: ER2005-0919

Mr. James Bearzi
NMED – Hazardous Waste Bureau
2905 Rodeo Park Drive East, Building 1
Santa Fe, NM 87505-6303

RECEIVED DEC 2005

SUBJECT: RESPONSE TO THE NOTICE OF DISAPPROVAL FOR THE REMEDY COMPLETION REPORT FOR THE INVESTIGATION AND REMEDIATION OF CONSOLIDATED UNIT 19-001-99 (FORMER TA-19/EAST GATE LABORATORY)

Dear Mr. Bearzi:

Enclosed please find two hard copies with electronic files of the response to the notice of disapproval for the "Remedy Completion Report for the Investigation and Remediation of Consolidated Unit 19-001-99 (former TA-19/East Gate Laboratory)."

If you have questions, please contact Becky Coel-Roback (505) 665-5011 (becky_cr@lanl.gov) or Robert Enz at (505) 667-7640 (renz@doeal.gov).

Sincerely,

David McInroy, Deputy Program Director
Environmental Remediation & Surveillance
Los Alamos National Laboratory

Sincerely,

David Gregory, Federal Project Director
Department of Energy
Los Alamos Site Office

BCR/jk

Enclosures: 1) Two hard copies with electronic files – Response to the Notice of Disapproval for the Remedy Completion Report for the Investigation and Remediation of Consolidated Unit 19-001-99 (former TA-19/East Gate Laboratory) (ER2005-0891)

1606

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Response to the Notice of Disapproval for the Remedy Completion Report for the Investigation and Remediation of Consolidated Unit 19-001-99 (Former TA-19/East Gate Laboratory) Los Alamos National Laboratory EPA ID No: NM0890010515 HWB-LANL-05-011

INTRODUCTION

This submittal is the response by Los Alamos National Laboratory (the Laboratory) to the "Notice of Disapproval for the Remedy Completion Report for the Investigation and Remediation of Consolidated Unit 19-001-99 (Former TA-19/East Gate Laboratory)" issued by the New Mexico Environment Department (NMED) Hazardous Waste Bureau on October 31, 2005, and received by the Laboratory on November 2, 2005.

NMED's comments are included verbatim and are divided into general and specific categories, as presented by NMED. The Laboratory's responses follow NMED comments. This response also contains the following attachment:

- Attachment 1 contains manufacturer's information on the polynuclear aromatic hydrocarbon (PAH) immunoassay test kit used for the field screening of PAH chemicals in soil.

This response includes information on radioactive materials, including source, special nuclear, and byproduct material. The management of these materials is regulated under the Atomic Energy Act and is specifically excluded from regulation under the Resource Conservation and Recovery Act and the New Mexico Hazardous Waste Act. Information on radioactive materials and radionuclides, including the results of sampling and analysis of radioactive constituents, is voluntarily provided to NMED in accordance with U.S. Department of Energy policy.

GENERAL COMMENTS

NMED Comment

1. *The Report provided a screening evaluation of human health and ecological risks to determine whether remedial action is warranted at the site. As part of an initial screening evaluation, the maximum detected site concentration is typically first used and not the 95% upper confidence level (95% UCL) on the mean. If the maximum detected site concentration exceeds a Soil Screening Levels (SSL), then additional analyses are conducted and the 95% UCL is then used as the exposure point concentration. It is noted that according to the "Screening-Level Ecological Risk Assessment Methods, Revision 2" (LA-UR-04-8246/ER2004-0519) either the maximum or the 95% UCL may be used, depending on sample size and spatial distribution. As there are concerns over the nature and extent of contamination at the site, a comparison of the site maximum detected concentrations to the SSL would be helpful in addressing uncertainties with characterization. The Permittees must provide a table comparing the maximum site concentrations to the appropriate SSLs. In addition, for future screening assessments, the site maximum detected concentrations are preferred as the first step in screening.*

LANL Response

1. The intent of the risk assessment, whether it is a screening assessment or a baseline assessment, is to evaluate the reasonable maximum exposure to a receptor or set of receptors. The use of the maximum concentration does not fulfill this objective; it is an assessment of the worst-case exposure. For human health assessments, the NMED guidance for use of SSLs states that "or, if deemed appropriate by NMED, the 95% UCL values" may be used for comparison. For the ecological assessment, the Consent Order requirement is to use the referenced guidance document, i.e., The Laboratory's "Screening Level Ecological Risk Assessment Methods," which calls for the 95% UCL to be used when appropriate. The 95% UCLs have been calculated and used in the assessments over the last 7–8 years whenever there are sufficient data to calculate a representative concentration. The continued use of 95% UCLs is appropriate, given the restrictions provided in EPA Risk Assessment Guidance for Superfund, Part A, i.e., a sufficient number of samples are collected, and the 95% UCL is not above the maximum concentration. At TA-19, the sampling was designed to adequately characterize the site and provide a sufficient data set to calculate representative 95% UCLs. Therefore, no additional assessments or presentations of data and SSLs in the revised report are necessary.

NMED Comment

2. *The Report indicates that one of the land uses is for recreational activities. However, the Report does not address comparison of site data to the Los Alamos National Laboratory recreational screening levels ("Draft Technical Approach for Calculating Recreational Soil Screening Levels for Chemicals" LA-UR-04-7743/ER2004-0610) nor does the Report provide a comparison of recreational levels to residential levels. If a specific comparison to recreational levels is not to be conducted, then the Permittees should provide a discussion indicating that the residential screening levels are more conservative than the recreational levels, and therefore, the risks to the recreationist would be less than those estimated for the resident. The Permittees must revise the Report to provide this analysis and/or discussion.*

LANL Response

2. The objective of the accelerated corrective action, as stated in the approved work plan, is to obtain approval from NMED that TA-19 is "complete without controls." The only scenario under which this approval can be obtained is for the potential risk associated with a residential scenario (the basis for the site decision) to be acceptable. NMED guidance states that "onsite residential receptors are expected to be the most conservative receptor basis for risk assessment purposes due to the assumption that exposure occurs 24 hours a day, 350 days per year, extending over a 30-day exposure duration." Therefore, any other scenarios, specifically recreational in the case of TA-19, are less stringent and do not need to be assessed, i.e., the residential scenario is protective of recreational users. If the "complete without controls" determination (based on a residential scenario) is approved, the site is suitable for any land-use scenario. Therefore, no revision to the report is necessary.

NMED Comment

3. *It is noted that screening levels were applied from primarily two sources, including the SSL for NMED and the Environmental Protection Agency (EPA) Region 6 SSLs. It is noted that the most conservative SSL was not always applied. For example, for polychlorinated biphenyls (PCBs), the Region 6 SSL is 2.22 mg/kg while the NMED SSL is 1.12 mg/kg. However, a comparison to both the*

NMED and Region 6 SSL indicated that the exposure point concentrations were below both values. In the future, the more conservative of the Region 6 or the NMED SSLs (or other screening levels applied) should be used. Please note this for future evaluations.

LANL Response

3. According to the Consent Order, NMED SSLs are used for all comparisons unless no SSL is provided for a chemical, in which case EPA Region-6 values are used. This is the methodology used for the screening assessments at TA-19, and the sources of the values are footnoted in each table. In all cases, the lowest SSLs were used for comparisons at TA-19. NMED guidance states that "In instances where an individual contaminant has the capacity to elicit both types of responses, the SSLs preferentially report the screening values representative of the lower (most stringent) contaminant concentration in environmental media." In the case of the PCBs at TA-19 noted in NMED's comment, the carcinogenic SSL of 2.22 mg/kg provided in NMED guidance was used in the comparison for the Aroclors (Table E-3.1-2). This carcinogenic SSL was also applied to Aroclor-1254, even though it is not explicitly presented as the SSL in NMED guidance; the lowest SSL for Aroclor-1254 is 1.11 mg/kg based on noncarcinogenic effects. The 1.11 mg/kg SSL was applied to Aroclor-1254 according to NMED guidance in the noncarcinogenic screening in Table E-3.1-3. Therefore, the most conservative/stringent SSLs were applied according to NMED guidance in the risk assessment, and no revisions to the report are necessary.

SPECIFIC COMMENTS

NMED Comment

1. *Section 2.5.3.1 Inorganic Chemical Data, pg. 9:*

   The Permittees have eliminated calcium and magnesium as contaminants of potential concern (COPCs) because they are essential nutrients. While studies have indicated that calcium is relatively non-toxic, studies have shown there to be an upper intake limit for magnesium. The United States Department of Agriculture Food Safety and Inspection Service and the National Academy of Science Food and Nutrition Board have developed upper intake levels (ULs) which should be applied in determining a soil screening level (SSL) that should be used in assessing essential nutrients toxicity. If site concentrations of magnesium are below the SSL, they may be eliminated from further consideration in the risk assessment. The Permittees shall revise the risk assessment accordingly.

LANL Response

1. Calcium and magnesium do not meet the definition of "contaminant" under the Consent Order and are not subject to the Consent Order. Furthermore, there is no human health toxicity factor for magnesium. The suggested upper intake level for magnesium is for the consumption of food, and does not apply directly to incidental ingestion of soil; the upper intake level is not representative of a toxic level. Therefore, no revisions to the risk assessments are necessary.

   In the specific case of TA-19, only calcium was detected above background value (BV) in the investigation samples collected according to the approved work plan. Magnesium was not detected above the BVs in the investigation samples (Table 4.2-4). Therefore, it is not a chemical of potential concern at TA-19.

Magnesium was detected above its BV in the historical data. However, these data were not used in the risk assessments. In the historical data, magnesium was detected above BV at concentrations of less than two times the maximum background concentration in the tuff (2820 mg/kg vs 4390 mg/kg). Therefore, based on EPA Risk Assessment Guidance for the Superfund, Part-A guidance, magnesium can be eliminated as an essential nutrient because it is only slightly elevated above background (i.e., less than twice the background concentration).

NMED Comment

2. *Section 4.2.2.1 PAH Screening by Immunoassay, pg. 18:*

   As stated in the approval letter for the work plan, the Permittees were to provide additional information on the PAH field test kit. The information was to include calibration procedures, and the amount, type, and frequency of quality control samples suggested by the test kit's manufacturer. The Permittees must provide this information.

LANL Response

2. Information on the PAH field text kit from the manufacturer, including calibration and quality control procedures, is provided as Attachment 1.

NMED Comment

3. *Section 4.2.4 Nature and Extent, Inorganic COPCs, pg. 22:*

   The Permittees have not offered an explanation for the elevated cobalt and chromium detections on the mesa top and south slope of the site at depth (between 4 and 6.5 feet). The fact that chromium is not detected above 4 feet does not reasonably suggest that the chromium below this depth is not the result of undocumented or unknown historical operations at TA-19, as the Permittees claim in this section. The fact that chromium and cobalt are increasing with depth does not support the Permittees' statement that "the extent of inorganic chemical contamination has been defined for this CU." The chromium levels are increasing with depth at five locations (19-22608, 19-22615, 19-22627, 19-22630, and 19-22631). Cobalt levels are also increasing with depth at these same locations. This suggests a release may have occurred from the former buildings or another contamination source exists that has not been identified. The Permittees did not determine the extent of these contaminants at these locations before performing the risk assessment. The Permittees must resample theses locations to determine the extent of contamination and, if the additional data warrant, revise the risk assessment using a residential scenario based upon a depth of ten feet below ground surface.

LANL Response

3. The elevated chromium and cobalt concentrations on the mesa top and on the south slope are not indicative of releases resulting from historical operations, but rather are indicative of subsurface geochemical anomalies. For both chromium and cobalt, the mesa-top locations with concentrations above background at depth do not have corresponding elevated concentrations between the surface and several feet of tuff. Surface and near-surface contamination would be expected at these locations if the elevated subsurface concentrations were a result of site operations because the buildings and associated pipelines were present only on the surface at the site. The elevated concentrations of chromium and cobalt, if a result of historical operations or releases, would also be expected at shallower depths. Additionally, the presence of chromium and cobalt on the southern edge and slope

of the site are not consistent with the historical operations and known releases at the site—all utility outfalls and debris disposal occurred only on the north slope of the site.

Despite the presence of the geochemical anomalies, additional samples will be collected from the mesa-top locations with chromium and cobalt concentrations above background (locations 19-22608, 19-22615, and 19-22627). Two samples from depths beyond the previously sampled depths will be collected at each location and analyzed for target analyte list metals. Additional samples cannot be collected from the south slope due to the extreme topography on the slope, concerns over safe access by the sampling team, and equipment limitations (as discussed between Terry Rust and Darlene Goering on November 16, 2005). Potential risk to on-site receptors will be reevaluated , as appropriate, upon receipt of the additional data collected at the site.

NMED Comment

4. *Section E-2.1 Historical Analytical Data, pg. E-5:*

   The discussion of historical data indicates that Cesium-137 (Cs-137) and Europium-152 (Eu-152) were detected in soil and/or tuff. However, it is not clear that these radionuclides were evaluated in the risk assessment, as these radionuclides were not addressed in the tables or plots comparing site data to background. The Permittees must discuss the detected site concentrations in relation to background. If warranted, the Permittees must revise the risk assessment to address these radionuclides.

LANL Response

4. Cesium-137 and europium-152 were identified as COPCs in the historical data from TA-19, which were only used to develop the sampling plan for the accelerated corrective action; these data were not used in the risk assessments. Some of the samples collected during the accelerated corrective action were analyzed for gamma spectroscopy, in accordance with the approved work plan. These data found that cesium-137 and europium-152 were not detected above the fallout value and therefore are not chemicals of potential concern at TA-19. As a result, these radionuclides were not evaluated in the risk assessments. Therefore, no revisions to the risk assessments are necessary.

NMED Comment

5. *Table E-3.1-2 CU 19-001-99 Carcinogenic Screening Evaluation, pg. E-38*

   This table presents the carcinogenic screening evaluation for the constituents of concern. However, several chemicals with carcinogenic toxicity have been omitted, including beryllium, cadmium, and cobalt. Many chemicals exhibit both carcinogenic and noncarcinogenic toxicity, and both toxicities must be evaluated in a risk screen. The Permittees must either provide justification for excluding these metals from the carcinogenic screening or revise the screening to include these metals.

LANL Response

5. The principle of a screening assessment is that the most stringent/toxic value is used as the point of comparison and that no additional assessment beyond this comparison is warranted if potential risk is not indicated. NMED guidance states that "In instances where an individual contaminant has the capacity to elicit both types of responses, the SSLs preferentially report the screening values representative of the lower (most stringent) contaminant concentration in environmental media." The

same is true for EPA Region 6 SSLs and guidance. NMED and EPA Region-6 guidance does not state or require that both endpoints must be evaluated in a screening assessment. In the case of beryllium, cadmium, and cobalt, NMED guidance provides only one SSL (the most stringent), which includes the values used in the screening assessment for TA-19. Therefore, no revisions to the risk assessment are necessary.

NMED Comment

6. *Table E-3.1-3 CU 19-001-99 Noncarcinogenic Screening Evaluation, pg. E-39:*

   This table presents the noncarcinogenic screening evaluation for the constituents of concern. However, several chemicals with noncarcinogenic toxicity have been omitted, including arsenic, chromium, bis(2-ethylhexyl)phthalate, isophrone, DDT, and dieldrin. Many chemicals exhibit both carcinogenic and noncarcinogenic toxicity, and both toxicities must be evaluated in a risk screen. The Permittees must either provide justification for excluding these metals from the noncarcinogenic screening or revise the screening to include these metals.

LANL Response

6. The principle of a screening assessment is that the most stringent/toxic value is used as the point of comparison and that no additional assessment beyond this comparison is warranted if potential risk is not indicated. NMED guidance states that "In instances where an individual contaminant has the capacity to elicit both types of responses, the SSLs preferentially report the screening values representative of the lower (most stringent) contaminant concentration in environmental media." The same is true for EPA Region-6 SSLs and guidance. NMED and EPA Region-6 guidance does not state or require that both endpoints must be evaluated in the screening assessment. In the case of arsenic, chromium, bis(2-ethylhexyl)phthalate, isophorone, DDT, and dieldrin, NMED/EPA Region 6 guidance provides only one SSL (the most stringent), which includes the values used in the screening assessment. Therefore, no revisions to the risk assessment are necessary.

NMED Comment

7. *Table E-3.1-3 CU 19-001-99 Noncarcinogenic Screening Evaluation, pg. E-39:*

   This table lists the SSL for Uranium-235 (U-235) as 17 mg/kg. However, in reviewing "Derivation and Use of Radionuclide Screening Action Levels, Revision 1" (LA-UR-05-1849/ER2005-0127), the screening action level for U-235 is in units of pico Curie per gram (pCi/g) and not mg/kg. Therefore, the application of the SSL of 17 mg/kg is not correct. Using a basic conversion equation, 0.15 pCi/g of U-135 is equivalent to 1 mg/kg U-235. Therefore, the SSL in units of mg/kg should be 0.26 mg/kg. The 95% UCL for U-235 is listed as 0.26 mg/kg, which is essentially equal to the SSL. Therefore, U-235 does not appear to be present at levels above acceptable risk limits. However, the Permittees must verify the SSL for U-235 and verify the units for the screening level.

LANL Response

7. As noted in the next to last row of Table E-3.1-3, the radionuclide data, specifically for uranium-235, are in units of pCi/g, not mg/kg. The SAL for uranium-235 is also in pCi/g; 17 pCi/g, not 17 mg/kg, as indicated in the NMED comment. Therefore, no revisions to the table or text are necessary.

   In addition, the specific activity of uranium-235 is 2.16×10^{-6} Ci/g, so 1 mg/kg is equal to 2.16 pCi/g.

Attachment 1

PAH Immunoassay Test Kit Information

DETERMINATION OF POLYNUCLEAR AROMATIC HYDROCARBONS (PAHs) IN SOIL AND WATER BY A MAGNETIC PARTICLE-BASED ENZYME IMMUNOASSAY SYSTEM.

Fernando M. Rubio, Timothy S. Lawruk, Charles E. Lachman and David P. Herzog, Ohmicron Environmental Diagnostics, 375 Pheasant Run, Newtown, Pennsylvania 18940; James R. Fleeker, North Dakota State University, P.O. Box 5516, Fargo, North Dakota 58105

Presented at the Fourth International Symposium on Field Screening Methods for Hazardous Wastes and Toxic Chemicals, Tropicana Hotel, Las Vegas, Nevada, February 22-24, 1995.

ABSTRACT

Use of immunoassays as field-screening methods to detect environmental contaminants has increased dramatically in recent years. Immunochemical assays are sensitive, rapid, reliable, cost-effective and can be used for lab or field analysis. A magnetic particle-based immunoassay system has been developed for the quantitation of Polynuclear Aromatic Hydrocarbons (PAHs) in soil and water. Paramagnetic particles used as the solid-phase allow for the precise addition of antibody and non-diffusion limited reaction kinetics. The magnetic particle-based immunoassay is ideally suited for on-site investigation and remediation processes to delineate PAH contamination. This system includes easy-to-use materials for collection, extraction, filtration and dilution of soil samples prior to analysis by immunoassay. When analyzing water samples, a simple dilution of the sample with methanol is performed during sample collection. The method detects PAHs, including anthracene, chrysene, fluoranthene, phenanthrene, pyrene and benzo[a]pyrene, at sub-parts-per-million levels in soil and at less than 1 ppb in water. The typical precision of the assay (within assay) in soil and water is less than 15% and 12%, respectively. Recovery studies (based on phenanthrene) from soil averaged 108%, and 107% from water. The analysis of soil samples by this ELISA correlate well with Method 8310, yielding a correlation coefficient (r) of 0.963; when water samples were compared to method 8270, a correlation (r) of 0.987 was obtained. The application of this ELISA method permits the cost-effective evaluation of samples with minimal solvent disposal and can result in savings of time and money. The system's flexibility allows the analysis of PAHs in many other sample matrices with minimum sample preparation.

INTRODUCTION

Polycyclic or polynuclear aromatic hydrocarbons (PAHs) are a group of compounds composed of two or more fused aromatic rings. The U.S. EPA has selected 16 unsubstituted PAHs as Consent Decree priority pollutants for regulatory purposes. Some of the four, five and six-ring PAHs such as chrysene, benzo[a]pyrene and indeno[1,2,3-cd]pyrene are considered to be possible or probable human carcinogens (U.S. EPA, 1985). The two and three-ring PAHs such as naphthalene, anthracene, and phenanthrene are considered non-carcinogenic and found as a component of certain grades of fossil fuels.

PAHs are introduced into the environment as a product of natural and fossil fuel combustion. Volcanic eruptions and forest fires are among the major sources of naturally produced PAHs. However, activities attributed to fossil fuel combustion sources, such as automobiles, coking plants, asphalt production, and manufacturing facilities that use fossil fuels, have dramatically increased the quantity of PAHs in the environment. Wood preserving sites that use creosote as a preservative, petrochemical

waste disposal sites, and leaking underground fuel storage tanks, have also contributed to the widespread contamination of PAHs in the environment.

The large number of sites contaminated with PAHs in soil and groundwater and the reenactment of key environmental legislation (Safe Drinking Water Act, Superfund Amendment Reauthorization Act) has led federal and state agencies to mandate clean-up. Federal and state agencies have set various regulatory levels for PAHs in soil; however, the usual concentrations of interest are 1 ppm and 10 ppm. In groundwater, the levels of interest are usually below 100 ppb. The analysis of PAH contamination in environmental samples is typically performed by GC/MS or HPLC methods, which are accurate and precise but can be time-consuming and expensive. This poster describes a magnetic-particle solid-phase immunoassay method for the analysis of PAHs in soil and water samples. Immunoassays have the advantage of being rapid and less expensive than GC/MS or HPLC, as well as field-portable.

The principles of enzyme linked immunosorbent assays (ELISA) have been described (Hammock and Mumma, 1980). Magnetic particle-based ELISAs have previously been described and applied to the detection of pesticide residues (Itak et al, 1993; Lawruk et al, 1993; Itak et al, 1992; Lawruk et al, 1992; Rubio et al, 1991). These ELISAs eliminate the imprecision problems that may be associated with antibody coated plates and tubes (Harrison et al, 1989; Engvall, 1980) through the covalent coupling of antibody to the magnetic particle solid-phase. The uniform dispersion of particles throughout the reaction mixture allows for rapid reaction kinetics and precise addition of antibody. The PAH magnetic-based ELISA described in this paper combines antibodies specific for PAHs with enzyme labeled PAHs. The presence of PAHs in a sample is visualized through a colorimetric enzymatic reaction and results are obtained by comparing the color in sample tubes to those of calibrators.

MATERIALS AND METHODS

Amine terminated superparamagnetic particles of approximately 1 um diameter were obtained from Perseptive Diagnostics, Inc. (Cambridge, MA). Glutaraldehyde (Sigma Chemical, St. Louis, MO). Rabbit anti-PAHs serum and PAH-HRP conjugate (Ohmicron, Newtown, PA). Hydrogen peroxide and TMB (Kirkegaard & Perry Labs, Gaithersburg, MD). PAHs and related compounds, as well as non-related cross-reactants (Chem Service, West Chester, PA).

The anti-PAH coupled magnetic particles were prepared by glutaraldehyde activation (Rubio et al, 1991). The unbound glutaraldehyde was removed from the particles by magnetic separation and washing four times with 2-(N-morpholino) ethane sulfonic acid (MES) buffer. The PAH antiserum and the activated particles were incubated overnight at room temperature with agitation. The unreacted glutaraldehyde was quenched with glycine buffer and the covalently coupled anti-PAH particles were washed and diluted with a Tris-saline/gel preserved buffer.

The various PAH compounds used during cross-reactivity studies were diluted in DMF to obtain a stock concentration of 1 mg/mL. The stock was further diluted in PAH diluent to obtain concentrations of 10 , 1, 0.1, 0.01, 0.001, and 0.0001 ppm. The creosote sample was diluted in methanol to obtain a stock concentration of 1 mg/mL; the stock was further diluted as described previously. After dilution, the diluted compounds were analyzed as samples in the assay.

When analyzing soil samples, a simple extraction was performed prior to analysis: 10 g of soil and 20 mL of a methanolic solution are added to a soil collector (Figure 1). The collector was shaken vigorously for 1 minute and the mixture allowed to sit at least five minutes. The cap of the soil collector was then replaced with a filter cap and the extract collected in a small glass vial. The filtered extract was then diluted 1:50 in PAH zero standard and assayed.

Water samples were collected in glass vessels with teflon lined caps. Immediately upon collection, samples were diluted with methanol 1:3 (1 part methanol:3 parts sample) to prevent adsorptive losses to the glass containers.

Diluted soil extract or water samples (250 uL) and horseradish peroxidase (HRP) labeled PAH (250 uL) were incubated for 30 minutes with the antibody coupled solid-phase (500 uL). A magnetic

field was applied to the magnetic solid-phase to facilitate washing and removal of unbound PAH-HRP and eliminate any potential interfering substances. The enzyme substrate (hydrogen peroxide) and TMB chromogen (3,3',5,5'-tetramethyl benzidine) were then added and incubated for 20 minutes. The reaction was stopped with the addition of acid and the final colored product was analyzed using the RPA-I RaPID Analyzer™ by determining the absorbance at 450 nm. The observed absorbance results were compared to a linear regression line using a log-logit standard curve prepared from calibrators containing 0, 2, 10, and 50 ppb of phenanthrene. If the assay is performed in the field (on-site), a battery powered photometer such as the RPA-III™ can be used.

RESULTS AND DISCUSSION

Figure 2 illustrates the mean standard curve for the PAH calibrators collected over 30 runs; error bars represent two standard deviations (SD). This figure shows the typical response of the assay and the reproducibility of the standard curve from run-to-run. The displacement at the 2 ppb level is significant (81.3% B/Bo, where B/Bo is the absorbance at 450 nm observed for a sample or standard divided by the absorbance at the zero standard). The assay sensitivity in diluent based on 90% B/Bo (Midgley et al, 1969) is 0.7 ppb. When analyzing water samples, the assay has a range of 0.9 to 67 ppb. The assay range when analyzing soils in conjuction with the PAHs Sample Extraction Kit is 0.2 to 5 ppm as a result of sample dilution.

A precision study was conducted using four surface and groundwater samples fortified with phenanthrene at four concentrations. The samples were diluted 1:3 with methanol and assayed 5 times in singlicate per assay on five different days. The results are shown in Table 1. Coefficients of variation (%CV) within and between day (Bookbinder and Panosian, 1986) were less than 12% and 10% respectively.

In another precision study, ten samples of two soils were weighed on a balance or measured by packed volume in the soil collector. The samples were then extracted and diluted (as described in the Methods Section), followed by assaying in duplicate in one assay. Results are shown in Table 2. The overall coefficient of variation for PAHs measurement using components of the Soil Collection and the PAHs Soil Extraction Kit with analysis by the PAHs RaPID Assay® was determined to be less than 18% in both cases.

Table 3 and Figure 3 summarize the cross-reactivity data of the PAHs RaPID Assay for various polynuclear aromatic hydrocarbons and petroleum products. The percent cross-reactivity was determined as the amount of analog required to achieve 50% B/Bo. The broad specificity of the antibody used, allows for the detection of a majority of the PAHs. Many non-structurally related organic compounds demonstrated no reactivity at concentrations up to 10,000 ppb (data not shown).

Table 4 summarize the accuracy of the PAHs RaPID Assay in soil samples. Thirteen different soil types were fortified with phenanthrene at 1 ppm. The samples were extracted and diluted as described above, followed by analysis in the immunoassay. The average recovery of phenanthrene in the samples was 108% with one sample (Alkali Lake) giving higher recoveries. The reason for the higher recovery on that sample is currently under investigation. To demonstrate the detection of other PAHs in soil, anthracene, benzo[a]pyrene, chrysene, fluoranthene, and pyrene, were spiked into four soils at 1 ppm; recoveries of those PAHs (data not shown) agreed closely with the predicted response based on the previously reported cross-reactivity data for the assay.

Table 5 summarizes the accuracy of the PAH ELISA in water. Four ground water samples were spiked with phenanthrene at the following levels: 5.0, 7.5, 20, and 40 ppb. Phenanthrene in the samples were recovered correctly in all cases with an average assay recovery of 107%.

Correlation of twenty-five samples, including both field contaminated soils and analytically spiked soils, analyzed by the ELISA method (y) and HPLC EPA Method 8310 (x) is illustrated in Figure 4. The regression analysis yields a correlation of 0.931 and a slope of 2.02 between methods. The lower values obtained with Method 8310 could be due to lower recoveries during the soxhlet extraction or to higher

values obtained by the ELISA due to >100% cross-reactivity of some of the PAHs in the ELISA system. Figure 5 illustrates the correlation of HPLC Method 8310 (y) versus the phenanthrene spike concentration (x) on 15 of the above soils; the regression analysis obtained was 0.991 with a slope of 0.49, indicating a bias with the HPLC method.

Figure 6 illustrates the correlation of 30 water samples between the ELISA (y) and Method 8270 (x). The regression analysis yields a correlation of 0.987 and a slope of 1.10 between methods.

SUMMARY

This work describes a magnetic particle-based ELISA for the detection of PAHs and its performance characteristics in soil and water samples. The assay compares favorably to HPLC or GC/MS determinations, is faster, and eliminates the need for expensive instrumentation and solvent disposal. The ELISA exhibits good precision and accuracy which can provide consistent monitoring of environmental samples. Using this ELISA, forty (40) results from soil samples can be obtained in less than two hours without the variability encountered with antibody coated tubes and microtiter plates (e.g. coating variability, antibody leaching, etc.). This system is ideally suited for adaptation to on-site monitoring of PAHs in water, soil, and solid waste samples.

REFERENCES

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Table 1

Precision of PAHs Measurement in Water

Pool Number	Pool 1	Pool 2	Pool 3	Pool 4
Replicates	5	5	5	5
Days	5	5	5	5
N	25	25	25	25
Mean (ppb)	5.9	8.9	24.4	44.1
% CV (within)	9.1	3.7	5.7	2.9
% CV (between)	13.8	11.5	8.8	8.1

Table 2

Precision of PAHs Measurement in Soil

Soil:	Wisconsin		Joshua Tree	
Sample Collection Method	weight	volume	weight	volume
Replicates	10	10	10	10
Mean (ppm)	1.57	1.18	1.43	1.26
% CV (total)	17.6	17.8	14.3	14.4

Table 3

Specificity (Cross-Reactivity)

Compound	90% B/Bo LDD (ppb)	50% B/Bo ED50 (ppb)	% Cross Reactivity
Phenanthrene	0.70	16.5	100
Anthracene	0.54	12.5	132
Fluoranthene	0.32	4.7	351
Chrysene	0.40	7.8	212
Pyrene	0.70	15.1	214
Benzo(a)Pyrene	0.50	6.9	239
Fluorene	1.65	35.2	47
Benzo(b)fluoranthene	0.91	54.2	30
Benzo(k)fluoranthene	0.77	524	3
1,12 Benzoperylene	14.7	>1000	<2
1,2:5,6 Dibenzanthracene	25.7	>1000	<2
Indeno (1,2,3-c,d)pyrene	0.78	27.2	61
Naphthalene	65	>1000	<2
Acenaphthalene	12.9	688	2
Acenaphthylene	10	447	4
1.2 Benzanthracene	0.77	28.4	58
1-Methylnaphthalene	28.2	1330	1.2
2-Methylnaphthalene	28.2	802	2.1
Aroclor 1242	37.5	1450	1.8
Aroclor 1248	41.0	5330	0.4
Aroclor 1254	>10000	>10000	<0.2
Aroclor 1260	>10000	>10000	<0.2
Benzene	>10000	>10000	<0.2
Toluene	>10000	>10000	<0.2
Phthalate	>10000	>10000	<0.2
Pentachlorophenol	340	>10000	<0.2
Biphenyl	15.9	703	2.8
CCA	>10000	>10000	<0.2
Creosote	1.1	16.6	117
Fuel Oil #6	5	53.7	31
Gulf Diesel Fuel	19.6	497	3
Sunoco Home Heating Fuel	12.8	292	6
Kerosine	1250	>10000	<0.1
Jet A Fuel	>10000	>10000	<0.1
Regular Gasoline	1000	>10000	<0.1
Premium Gasoline	597	>10000	<0.1

B/Bo = Absorbance at 450 nm observed for a sample or standard divided by the absorbance at the zero standard.

% Cross Reactivity = Concentration of phenanthrene exhibiting 50% inhibition (16.5 ppb) divided by the 50% inhibition of a compound x 100.

Table 4

Phenanthrene Recovery From Different Soil Types

Soil Sample	Neat (ppm)	Total (ppm)	Recovered (ppm)	Recovery (%)	Soil Type
Alkali Lake	0.50	2.19	1.69	169	loamy sand
Beardon	0.42	1.45	1.03	103	clay loam
Holland	2.30	3.22	0.92	92	silt loam
Joshua Tree	0.07	1.28	1.21	121	sand
Levittown	4.70	5.82	1.12	112	silt loam
Muck	2.90	3.94	1.04	104	organic potting
Munin	0.17	1.23	1.06	106	clay loam
Piscataway	2.69	3.67	0.98	98	sandy loam
Sagamore	4.40	5.56	1.16	116	silty clay loam
Sharkey	0.68	1.92	1.24	124	clay loam
Tennessee	0.30	1.29	0.99	99	sandy loam
Wiscosin	0.72	1.80	1.08	108	loam
Virginia	1.54	2.64	1.10	110	loamy sand
Mean			1.12	108	
SD			0.19		
%CV			17.1		

Neat = background concentration of a sample
Total = Observed concentration after fortification with 1 ppm phenanthrene
Recovered = Total - Neat

Table 5

Phenanthrene Recovery In Water

Added (ppm)	Observed (ppm)	SD (ppm)	Recovery (%)
+ 5.0	5.4	0.49	109
+ 7.5	8.2	0.74	109
+ 20	22.3	2.12	112
+ 40	39.9	3.06	100
Average			107

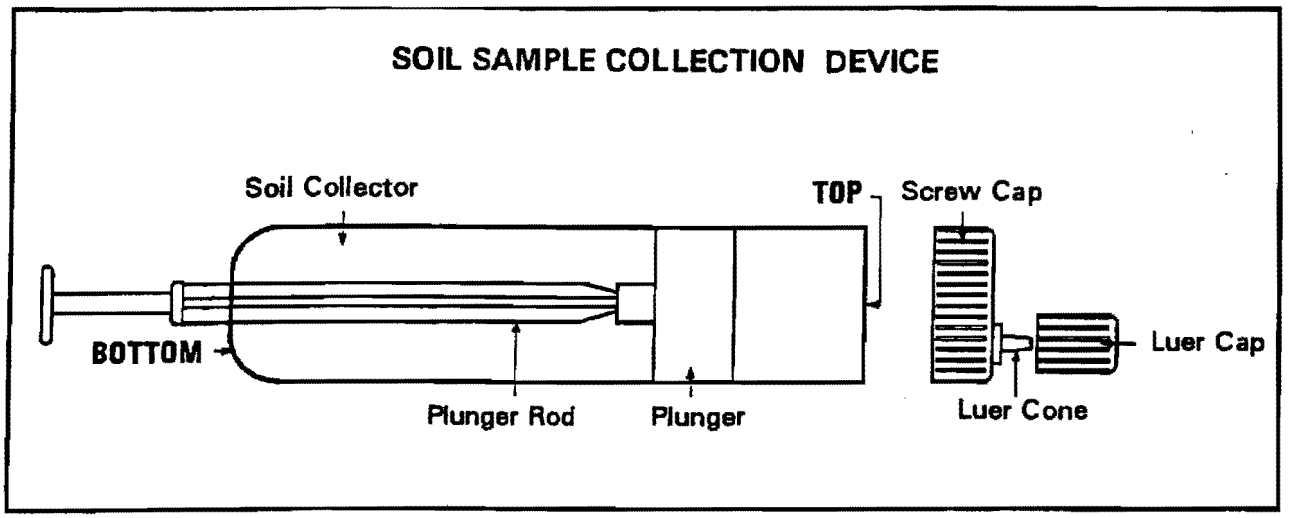

Figure 1. Diagram of soil collector used to collect and extract soil samples.

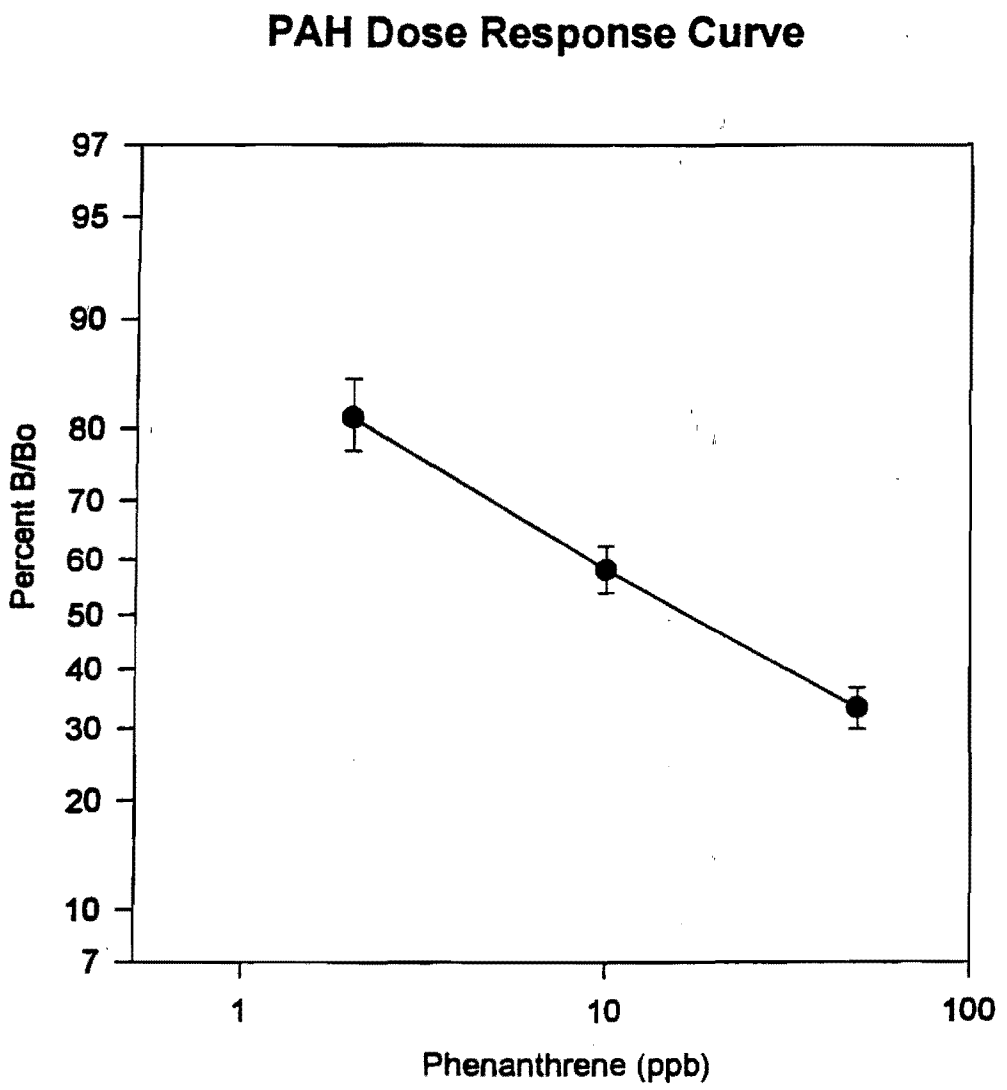

Figure 2. PAHs RaPID Assay dose response curve. Each point represents the mean of 30 determinations. Vertical bars indicate +/- 2 SD about the mean.

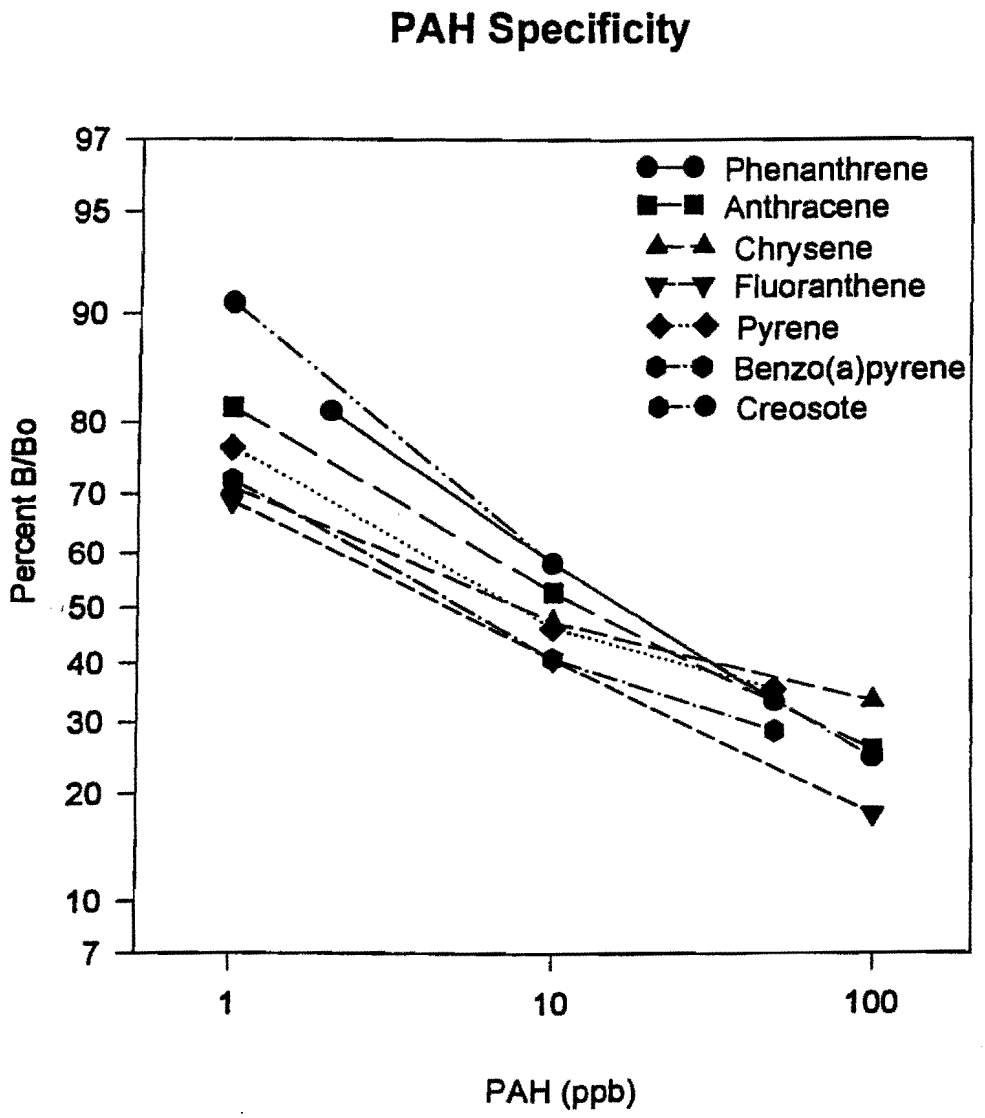

Figure 3. Specificity of the PAHs RaPID Assay against selected polynuclear aromatic hydrocarbons and creosote.

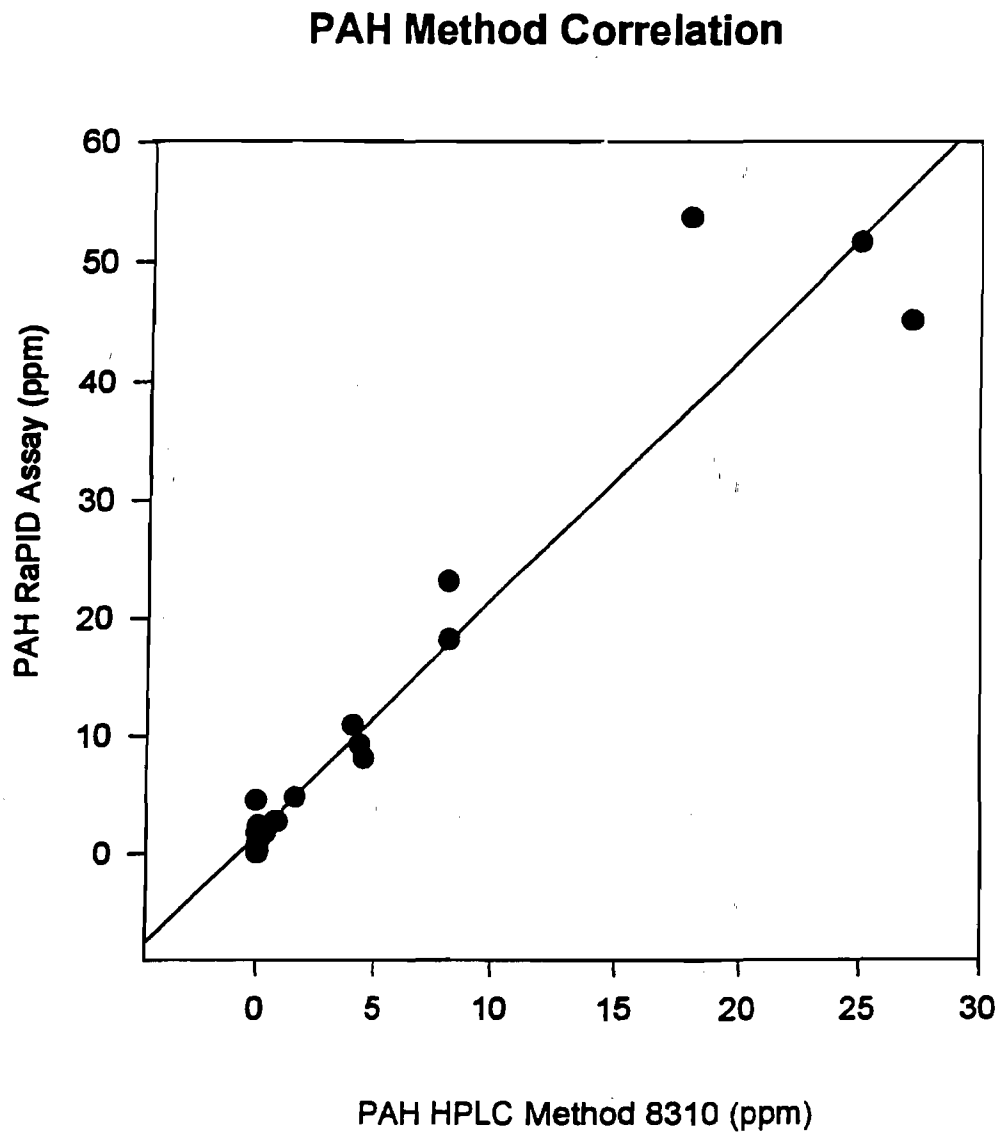

Figure 4. Correlation between PAHs concentrations as determined by the ELISA and HPLC Method 8310 in soil samples. $n = 25$, $r = 0.931$, $y = 2.02x + 1.55$.

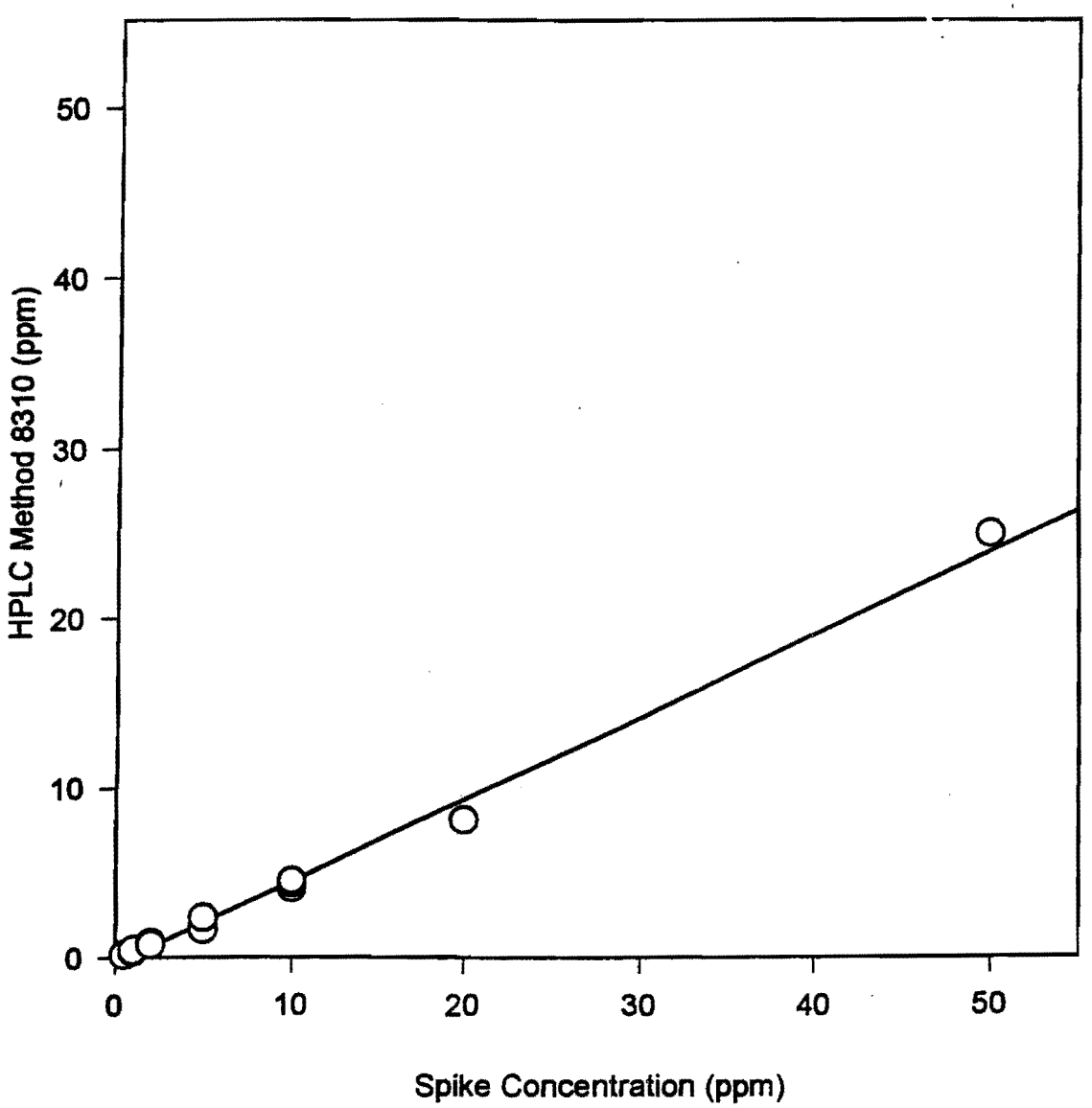

Figure 5. Correlation between PAHs concentrations as determined by HPLC Method 8310 vs. phenanthrene spiked concentration in soil samples. $n = 15$, $r = 0.991$, $y = 0.49x - 0.42$.

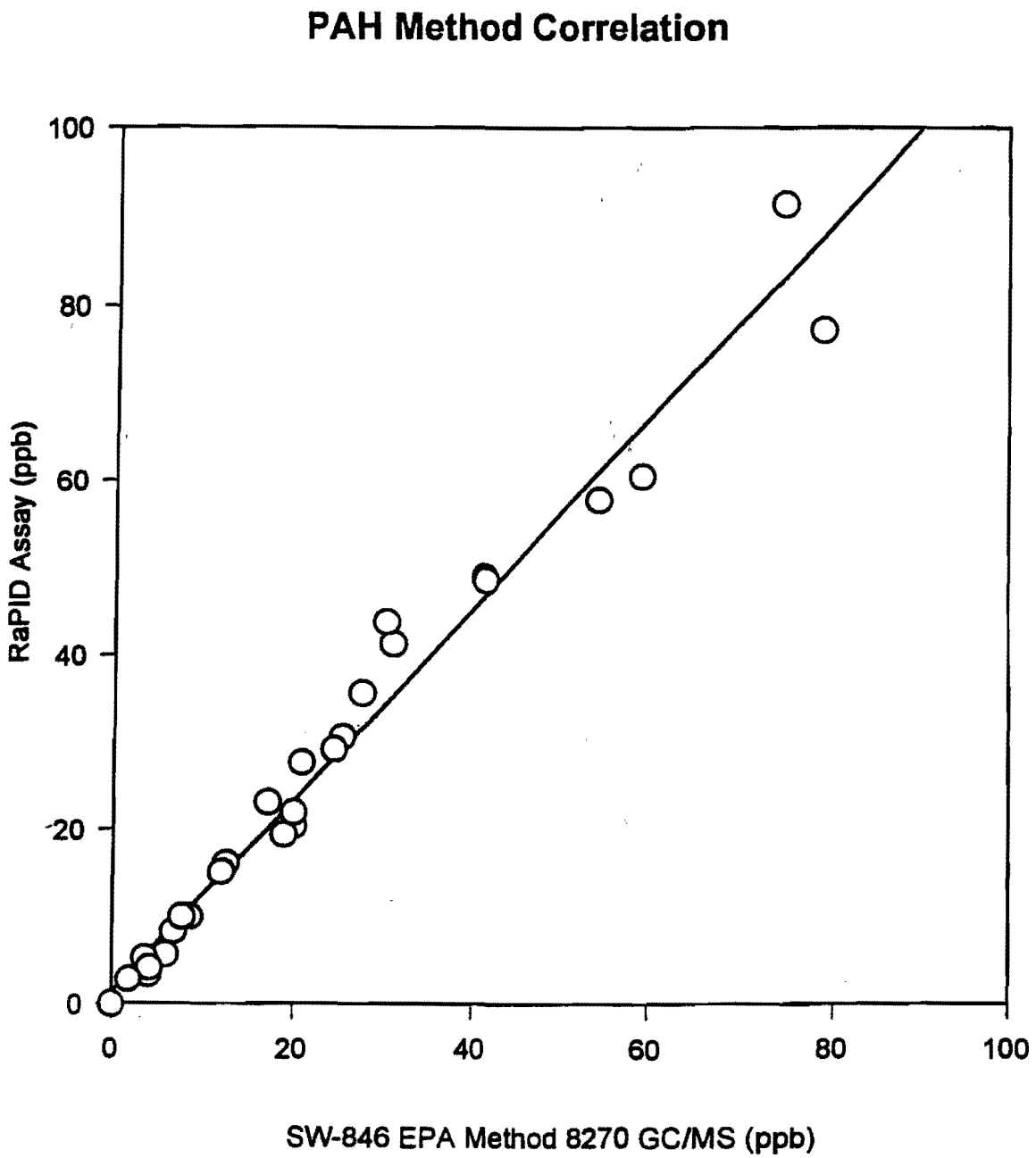

Figure 6. Correlation between PAH's concentrations as determined by the ELISA and GC/MS Method 8270 in water samples. n = 30, r = 0.987, y = 1.10x + 1.34.