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Development and Evaluation of a Quantitative Enzyme Linked Immunosorbent Assay (ELISA) for Polychlorinated Biphenyls

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by

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NOTICE

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ABSTRACT

A 96-well, microplate-based enzyme linked imunosorbant assay (ELISA) for the quantitative determination of PCBs (as Aroclors) in soil has been developed and evaluated. The assay detection limit for Aroclor 1248 in soil is 8.95 µg/Kg. The detection limit for Aroclor 1242 in soil is 10.5 µg/Kg. The assay has a linear dynamic range of 8 to 200 ng/mL in assay solution, corresponding to soil concentration ranges of 50 to 1330 µg/Kg after correction for dilution of soil extracts into the ELISA. Extracts of soil samples containing more than 1330 µg/Kg must be further diluted to bring them into the assay working range.

The assay was characterized for potential cross-reactivity using 37 structurally related compounds. Chlorinated benzenes, phenols and anisoles which might be present as co-pollutants in environmental samples were found to exhibit no more than 3% cross-reactivity relative to Aroclor 1248. Cross-reactivities less than 0.1% were the norm. In addition, insignificant cross-reactivity was observed for a number of environmentally occurring chlorinated pesticides.

The long term (6 month) relative standard deviation (RSD) for determination of Aroclor 1248 in soils was found to range from 5 to 10%, dependant upon concentration. The long term (3 month) RSD for determination of Aroclor 1242 was found to range from 5 to 30%, again dependant on concentration.

Sample preparation procedures for the quantitative PCB plate ELISA were developed by performing extraction recovery studies on samples spiked with C-14 radiolabeled tetrachloro biphenyl solutions. The samples were extracted with a simplified shake extraction procedure using methanol; the extraction provided greater than 90% extraction efficiency for samples spiked with C-14 radiolabeled tetrachloro biphenyl. Similar extraction efficiencies (as quantitated by ELISA) were obtained for seven PCB soil standard reference materials (SRMs).

Spike recovery (as quantitated by ELISA) for real-world samples ranged from greater than 80% to 143%, which complies with recovery requirements stated for EPA gas chromatography (GC) methods (e.g. SW-846 Method 8080/81 and the Contract Laboratory Program method). Recovery of spike solution from clean extraction solution gave a mean recovery of 101.9%, illustrating ELISA accuracy.

Effects of the sample matrix upon assay performance were examined for real-world samples by carrying out parallelism studies. Sets of serially diluted samples were simultaneously analyzed with the ELISA. The assay response, corrected for dilution, should provide equivalent results in the absence of matrix effects. Ten percent of the real-world samples were analyzed in this fashion. After arithmetic correction for dilution, the mean calculated PCB concentration for each group of sample replicates had RSD's no greater than 14% ($n \geq 3$). Because this variability is not significantly greater than assay variability, it did not appear that the matrix interfered with the assay.

Three sets of real-world samples, obtained from EPA Superfund Innovative Technology Evaluation (SITE) demonstrations, EMSL-LV Technical Support Center demonstrations and regulatory activities, were analyzed employing the PCB ELISA. Splits from 110 Aroclor 1248 contaminated clay samples were obtained from a SITE demonstration study at the abandoned Indian Creek outfall (AICO) at a Department of Energy plant in Kansas City, MO. The splits were analyzed by a participant in the Contract Laboratory Program (CLP) and by ELISA. The sample splits analyzed by

ELISA were extracted using the methanol shake procedure and a subset of randomly chosen splits was extracted using supercritical fluid extraction (SFE) employing CO_2.

From these samples 3 data sets were generated (Shake/ELISA, SFE-ELISA, and CLP). Shake/ELISA and the CLP data sets were judged to be non-equivalent by a paired t-test; the Shake/ELISA results were biased high, with a mean relative difference of 1.5. The SFE-ELISA results were found to be equivalent to the CLP results by paired t-test ($t = 0.8729$, $p = 0.39$). The SFE-ELISA results appeared to be biased low relative to the CLP results, with a mean relative percent difference (RPD) of -14%.

Samples were obtained from a technical support study conducted at the Allied Paper/Portage Creek/Kalamazoo River (APPC) Superfund site in Michigan consisting of Aroclor 1242 contaminated soil, sediment and paper pulp waste. Sample splits were analyzed by EPA SW-846 Method 8081 and by ELISA. The 39 sample splits analyzed by ELISA were extracted using the methanol shake extraction and Soxhlet extraction using methanol. Aroclor 1242 contamination ranged from non-detect at 0.5 mg/Kg to 268 mg/Kg by CLP analysis.

For samples below 30 mg/Kg, ELISA results were generally within 2 standard deviations of the Method 8081 results, whereas at the higher levels, results by ELISA were biased lower than the Method 8081 results by up to a factor of 6.5. ELISA analysis of the more vigorously extracted Soxhlet extracts from the higher level samples provided results which were within 2 standard deviations of the Method 8081 results. This demonstrates the need to evaluate the quantitative PCB plate ELISA as a separate determinative technique with bias from sample preparation removed.

An assortment of samples obtained from the EPA National Enforcement and Investigation Center (NEIC) were extracted with the shake extraction and analyzed by ELISA. Analysis by NEIC using gas chromatography indicated contamination with Aroclors 1242, 1254, and 1260. ELISA results were on average biased high, with a mean RPD of 37%. The quantitative PCB plate ELISA is approximately twice as sensitive to Aroclor 1254 relative to Aroclor 1242, thus the results are consistent with analysis of 1242/54/60 mixtures with 1242 calibration. Re-calibration with Aroclor 1254 would provide accurate quantitative results.

The quantitative PCB plate ELISA data tracked the confirmatory method data, although the ELISA results appeared to have a slight statistical bias away from the standard GC based methods. The quantitative PCB plate ELISA data for the three sets of real-world data contain more information than results which may be generated employing commercially available semi-quantitative ELISA-based methods, and thus, the quantitative PCB ELISA fulfills the goal of providing an easily performed method bridging the performance gap between GC methods and semi-quantitative ELISA. The data generated during the development, evaluation, and application of the quantitative PCB ELISA strongly suggest that the quantitative PCB ELISA can function in a highly accurate and precise manner as a "detection and quantitation device" when coupled to an efficient extraction procedure.

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SECTION 1

INTRODUCTION

This report details the development and evaluation of a 96-well microplate-based enzyme linked immunosorbent assay (ELISA) for the quantitative determination of polychlorinated biphenyls (PCBs) in soil. Procedures carried out during the developmental stage will be described, along with accompanying performance characteristic data. In addition, the analysis of three sets of real-world soil samples, representing a wide variety of matrix challenges, is reported.

After the initial detection of polychlorinated biphenyls (PCBs) in the environment in 1966 (Jensen et al., 1966), mounting evidence led to the United States Environmental Protection Agency (EPA) to classify them as suspected human carcinogens, due in part to their low rate of degradation, their tendency to bioaccumulate, and their carcinogenic nature. In 1976, the United States Congress banned PCB manufacture, processing, distribution and use, except for a handful of specific and limited uses. As a consequence, analytical method development resulted in the codification of a number of standard methods for PCBs. One such method, Method 8080, described in the EPA Office of Solid Waste and Emergency Response Manual SW-846 (U.S. Environmental Protection Agency, 1986), is representative of the majority of PCB analytical methods in that the method relies on rigorous overnight Soxhlet extraction, followed by gas chromatography (GC) for quantitation of PCBs.

Several factors have generated increasing interest in immunochemically based analytical methods, such as ELISA, for PCBs. In addition to the spiralling costs associated with regulatory compliance, current toxicological research has re-awakened the controversy surrounding the actual carcinogenicity and toxicity of PCBs. In an effort to expand the array of tools available for PCB research, immunochemically based methods are increasingly being relied upon to provide data under appropriate circumstances.

As a result of the Superfund Amendments and Re-authorization Act of 1986 (SARA), site monitoring and assessment will represent a large and possibly rising financial burden directly related to the collection, transport, and analysis of PCB containing samples. ELISA based methods have been shown to offer the potential for data outputs which are complimentary and in some case comparable to established methods at a significantly lower cost on a per analysis basis. ELISA methods also show a distinct advantage with regard to timeliness of data generation.

Several ELISA based methods for PCBs have become commercially available over the past several years. For example, Ohmicron Environmental Diagnostics, Inc., the Immunosystems division of Millipore, Inc., EM Science Inc., and Ensys, Inc., currently market tube-based immunoassays intended for field screening applications. In general, these immunoassays are formatted to be used only for determining whether a given sample contains PCBs at a concentration above or below a set threshold value, although the Ohmicron assay is intended for quantitative use. As such, these immunoassay kits are designed for rapid result generation, low cost, and ease of use by relatively untrained personnel. The benefits of such design criteria have become quite evident to the EPA, which, under the Superfund Innovative Technology Evaluation (SITE) Program, officially mandated the use of low cost, rugged, field portable methods to ease the burden of using expensive, time consuming, GC or GC/MS methods for the characterization of contaminated areas.

While the benefits of using commercial immunoassay kits are evident, the data which they generate is complimentary but not comparable with current GC-based methods for PCBs. Consequently, there exists a large gulf between these analytical methods.

In the broadest of terms, this report describes work on an immunoassay aimed at bridging this gap between the GC based instrumental methods and the commercial immunoassay kits. Such an immunoassay procedure will provide data which are quantitative and comparable to the GC based methods for many applications, such as site characterization, mapping concentration isopliths, and monitoring remedial activities, while providing high throughput analytical procedures which are to a high degree as inexpensive, rapid, and simple as the kit immunoassays.

SECTION 2

CONCLUSIONS

ASSAY PERFORMANCE

The quantitative PCB plate ELISA was characterized over the course of assay development and subsequent analysis of three sets of real-world samples obtained from EPA SITE demonstrations and regulatory activities. Based on the Aroclors which contaminated these real-world samples the bulk of data are focused on Aroclors 1242 and 1248. Initial characterization data show that this assay could be used for Aroclors 1254 and 1260 equally well with similar performance characteristics. The remainder of the discussion centers around Aroclors 1242 and 1248.

The assay described in the current study is intended for the analysis of PCB contamination in solid matrices such as soil, sediment, clays and paper pulp, and thus the samples required extraction prior to analysis. The methanol based shake extraction procedure employed during the present study was chosen for its simplicity, and was based on extraction procedures common to a number of field methods (U.S. Environmental Protection Agency, 1992a, 1992b). It was not the goal of the current study to develop extraction procedures per se, as this represents a potential research area in itself. Preliminary extraction studies, with a wide variety of matrices, using a radio-labeled tetrachloro biphenyl suggested that the extraction procedure would optimally provide an average extraction efficiency of 92% with a relative standard deviation (RSD) of variation of about 4%.

Extraction of commercially available "PCBs in soil" standard reference materials (SRMs), followed by quantitation with the plate ELISA provided an indirect measure of extraction efficiency. ELISA results for Aroclor 1248 SRMs suggest extraction efficiencies greater than 90%, while for Aroclor 1242, employing 5 PCB levels, efficiencies ranging from 53% to 91% were observed. In all cases for the 1242 SRMs, the reported value is within the EPA defined advisory range as specified by SW-846 Method 8080/81.

The assay had detection limits of 1.31 ng/mL for Aroclor 1248 with a σ of 0.9 ng/mL and a detection limit of 1.6 ng/mL for Aroclor 1242 with a σ value of 0.61 ng/mL. The detection limit in soil (based on a 5 gram sample) is 9.0 ng/g, σ = 6.0 ng/g for Aroclor 1248 and 10.5 ng/g, σ = 4.1 ng/g for Aroclor 1242 after correcting for the dilution factor imposed by adding soil extracts to assay solution. The assay had a quantitation range of about 8 ng/mL to 200 ng/mL in assay solution, corresponding to soil concentrations of about 50 ng/g to 1330 ng/g, or 0.05 mg/Kg to 1.33 mg/Kg. Samples extracts containing greater than about 1.3 µg/mL PCBs require appropriate dilution to bring the PCB concentration into the working range of the assay.

The assay provided the long-term reproducibility required for use as a quantitative tool. Based on repeated measures of Aroclor 1248 soil SRMs over a six month period, determinations were carried out with RSD's for all SRMs of less than 10%. Repeated measures of Aroclor 1242 soil SRMs over a three month period provided similar performance. Dependant on PCB level, RSD's ranged from 30% to 5%.

The quantitative PCB plate ELISA was found to be highly selective for PCBs; it exhibited very little cross-reactivity with a large number of compounds which might potentially co-contaminate environmental samples and interfere with accurate measurement of PCB concentrations. The assay

exhibited selectivity for PCBs which will allow the plate ELISA to be used in the presence of a wide range of commonly occurring chlorinated anisoles, benzenes and phenol co-pollutants. The 37 compounds which were studied in the preliminary development stage cross-react no more than about 3 percent relative to Aroclor 1248.

VALIDATION OF ELISA PERFORMANCE WITH REAL-WORLD SAMPLES

Validation of the ELISA using comparative results obtained by standard instrumental methods is based on several important assumptions. Statistically, this procedure can become confounded by sampling errors, sample preparation differences, and inter-lab variation, even before variability is introduced by true inter-method differences. Comparison of the quantitative PCB plate ELISA soil sample results with results generated using standard methods such as SW-846 Method 8080/81 is made difficult by the fact that performance data for the standard methods is typically limited to either solution phase measurement data or a limited number of soil matrices. This lack of availability of extensive soil analysis performance data for standard methods points out the difficulty of comparing results across soil samples; each soil matrix may present new challenges a particular method cannot meet. Extraction procedures which work well for sandy soils may provide irregular performance characteristics when applied to oily clay samples or sediments.

ELISA Analysis of Kansas City Samples

ELISA analysis of Aroclor 1248 contaminated clay samples obtained as sample splits from the Kansas City, MO Indian Creek Superfund site provided data which are interesting considering the above discussion. Using a paired t-test as the basis for decision, it was found that the PCB levels as reported by the CLP laboratory were not equivalent to the PCB levels as determined by the quantitative PCB plate ELISA. The average relative percent difference was found to be 46%.

One problem with such an approach is the implicit assumption that both the ELISA and CLP reported values represent the true mean for each respective method. The large error bars for the methods suggest that this is not likely to be the case. One undesirable alternative would be running enough replicates of each sample to ensure the validity of this assumption. This degree of rigor is possible with immunoassay, however, given the time and expense of the CLP analyses, this is not practical.

Another problem is the distinction (or lack thereof) between preparative and determinative steps in the data generation. The ELISA and CLP methods employed very different extraction procedures. It is conceivable that most of the measured difference between methods is due to sample preparation alone.

Four alternative hypotheses can explain the data. The first is: the quantitative PCB plate ELISA is not accurate but the CLP method is. The second is: Some interferant or interferants in the samples themselves causes assay performance degradation. The third hypothesis is: the quantitative PCB plate ELISA and CLP method are both accurate, but extraction performance varied greatly between methods. Finally, the fourth hypothesis is: there is a large quantity of some non-PCB cross-reacting species present in the samples which elevates the apparent concentration of PCBs as measured by the quantitative PCB plate ELISA.

The first hypothesis can be ruled out. Data generated for the commercial Soil SRMs as well as quantitation of spiked solutions shows that the ELISA can accurately measure PCB concentrations. The second hypothesis can be ruled out as well. Data collected during parallelism studies with the Kansas City samples show that there are no significant non-specific matrix contributions for the ELISA results.

The third hypothesis cannot be readily discounted. Based on earlier work (Spittler, 1986), it might be suspected that extraction procedures employing methanol would work better than hexane/acetone extractions as called for in the CLP method. Hexane/acetone extraction as specified in the CLP method may be optimal for a mixture containing all the chlorinated analytes covered by the method, but not for the specific, more focused use of PCB extraction exclusively. Of course, the simple approach taken in a methanol based shake extraction used for the ELISA might offset the gain realized from substitution of methanol. Results for the SFE extracts of the Kansas City samples illustrate these extraction issues. Using the identical ELISA procedure, it was found that the SFE extracts gave results which converged toward the CLP results. The SFE-ELISA results were equivalent to the CLP results by a paired t-test ($t = 0.8729$, $p = 0.39$), whereas the ELISA results for the same samples extracted by the methanol shake procedure were not ($t = 2.118$, $p = 0.046$).

The fourth hypothesis cannot be ruled out easily either. If there are cross-reacting compounds, they are not commonly occuring chloro benzenes, phenols, or anisoles. One possibility is the presence of PCB metabolites, such as polychlorinated biphenylols, which would not be detected by standard methods, but which may nevertheless be measured by ELISA.

There are certain applications for which data provided by the quantitative ELISA could prove very useful. The reported data for the Kansas City samples demonstrate the use of ELISA as a bridge between GC methods and semi-quantitative immunoassay test kits. The majority of the samples had concentrations well below 5 mg/Kg. At this level, relative percent differences of 100% may correspond to as little as 0.033 mg/Kg (the detection limit of the CLP method). For example, to easily obtain a quantitative PCB result of 0.1 mg/Kg, ± 0.1 may have great value when the option is either GC analysis or a semi-quantitative "less than 5 mg/Kg" result obtained through use of a commercial, semi-quantitative ELISA. The quantitative PCB plate ELISA allows for the measurement of PCB concentration in a way which provides more information than the semi-quantitative ELISAs currently available commercially, while using an assay procedure of similar complexity.

ELISA Analysis of Allied Paper/Portage Creek/Kalamazoo River Samples

ELISA analysis of Kalamazoo samples obtained from the Allied Paper/Portage Creek/Kalamazoo River Superfund site in Michigan provides further amplification upon the points discussed above. The data can be thought of as consisting essentially of two subsets, the low level samples (PCB concentrations below approximately 30 mg/Kg) and the high level samples (PCB concentrations greater than 30 mg/Kg). For the low level samples, the bulk of the ELISA and SW-846 Method 8081 results overlap one another within the 95% confidence intervals of the respective methods. For the high level samples, ELISA and Method 8081 results overlapped in a similar manner after more vigorous methanolic Soxhlet extraction prior to ELISA analysis. ELISA analysis of extracts obtained using a 20 minute shake in methanol resulted in measured values of PCB up to a factor of 6.5 lower than ELISA results for methanolic Soxhlet extracts. Again, parallelism studies and spike recovery data demonstrated that the quantitative PCB plate ELISA, as applied to the Allied Paper/Portage Creek/Kalamazoo River samples, showed high accuracy and no assay degradation due to

matrix artifacts. Thus, the potential utility of the quantitative PCB plate ELISA as a determinative method for PCBs in sediment, soil and paper waste was demonstrated by the results.

The results for the high concentration samples illustrate the need for differentiating the sample preparation from the determinative step itself. The ELISA results for the simple "20 minute methanolic shake" extracts and the ELISA results for the methanolic Soxhlet extracts are very different. Clearly, only the extraction efficiency plays a significant role in altering the ELISA results.

The fact that the ELISA results for the methanolic Soxhlet extracts are convergent with the CLP data (the ELISA results appear to be slightly lower than the CLP results with a calculated mean RPD of -17%) gives rise to the hypothesis that the quantitative PCB plate ELISA, as the determinative step, provided comparable data to the GC, for these environmental samples, provided that appropriate extraction procedures were used.

ELISA Analysis of NEIC Samples

The results from ELISA analysis of samples obtained from the EPA Enforcement and Investigation Center (NEIC) illustrate a number of points. Analysis of several serial dilutions of the sample extracts demonstrated that the assay was not subject to performance degradation due to matrix artifacts. The ELISA results were generally higher than the corresponding GC results, with an average RPD of 37%.

The results for this data set raise the issue of calibration, an issue which is universal to any determinative method for Aroclors. The samples were characterized by NEIC as being mixtures of Aroclors 1242/1254/1260. The apparent bias high on the part of the ELISA may be due wholly to selection of the calibration mixture. In addition, the PCB levels reported by NEIC most likely have a built in bias, due to analyst judgement calls on assigning peak patterns to the various Aroclors.

Taken as a complete method, 20 minute methanolic shake extraction followed by ELISA determination of PCBs appears to have bias away from standard GC based methods, at least statistically speaking. The quantative PCB plate ELISA data for the three sets of real-world samples contain more information than results which may be generated employing commercially available semi-quantitative ELISA-based methods. Thus, the quantitative PCB plate ELISA fulfills the goal of providing an easily performed method bridging the performance gap between GC methods and semi-quantitative ELISA.

The real-world data indicate that extraction procedures play a major role in the ELISA results. Statistically speaking, in these studies, it is improper to compare the accuracy of the ELISA determinative step with the GC determinative step, because the extraction procedures confound matters.

The data generated during development, evaluation, and application of the ELISA strongly suggest that the quantitative PCB ELISA can function in an accurate and highly precise manner when considered as a "detection and quantitation device" separate from the sample preparation itself.

SECTION 3

RECOMMENDATIONS

The analyses conducted in the course of the current study strongly suggest that the PCB ELISA has great potential for use as a determinative step in PCB analysis, in particular, when coupled with an appropriate sample preparation procedure. To ascertain the performance of the ELISA as a "detector system," it will be important to remove the statistical ambiguity resulting when the data being generated by two detectors (GC/electron capture detector and ELISA) are actually the result of measurements on two distinct soil extracts. The two soil extracts are very likely different in their PCB concentrations, and thus, even in the best case scenario of 100% accuracy in the measurement step, the results will of course differ.

To remove the contribution of errors in soil extraction, it is suggested that further experiments be carried out in which samples are extracted and portions of the extracts are quantitated by both GC and ELISA. Any extract cleanup procedures should be carried out before splitting the extract. Alternatively, a study design could be structured such that analyses could be carried out on extracts which had been cleaned up as well as extracts which had not been subjected to additional clean-up steps, thereby allowing for checks on the possible effects of the cleanup procedure.

Further work could be carried out to allow unambiguous comparison between the PCB ELISA and GC/ECD as quantitation techniques. One possible scenario would entail re-extraction of the Kansas City and/or Allied Paper samples, followed by analysis using ELISA and GC (in-house and/or CLP laboratory) of splits derived from these extracts.

The results of this study suggest that the ELISA has the capability of providing useful data for certain applications. The indirect inhibition format was used because, generally, it is one of the more sensitive formats which can be chosen from the myriad of ELISA formats. In addition, this format prevents exposure of the enzyme to potential interferants present in the original sample. The assay can be configured to allow even greater ease of use. For example, the equilibration times may be reduced allowing a one day assay without a substantial change in performance.

The plate ELISA format can be easily automated using any number of the readily available robotic plate ELISA instruments. This would permit screening of large numbers of samples, and in addition, it could allow for the carrying out of extensive parallelism studies on a routine basis. Extensive quality assurance of the ELISA data output could thus be ensured.

A note of caution is raised with respect to simple or abbreviated extraction procedures. Typically, most of the commercially available semi-quantitative ELISAs for soil screening rely upon "quick shake" extraction procedures, enabling extreme streamlining of the entire ELISA based procedure. The experiences noted in the current study reflect the possible dangers in assuming that these extractions always perform adequately.

The quantitative PCB plate ELISA can be used to measure PCBs with high accuracy and precision when coupled with appropriate sample preparation procedures. Further utilization should include coupling the quantitative PCB plate ELISA with efficient and potentially fieldable extraction methods, such as supercritical fluid extraction. Additionally, the quantitative PCB plate ELISA could be coupled with rapid Soxtec™ type extraction procedures, potentially in mobile laboratories, enabling

rapid, relatively non labor-intensive measurement of PCBs. This would be an analytical scheme of high utility, and acceptance, as use of Soxtec type extraction procedures for PCBs already has precedence in such methods as EPA SW-846 Method 3541, Automated Soxhlet Extraction (U.S. EPA, 1993).

Interest in the application of the quantitative PCB plate ELISA has been generated within a number diverse groups, such as the EPA Great Lakes National Program Office and the Fish and Wildlife Service, National Fisheries Contaminant Research Center. In order to facilitate the application of the quantitative PCB plate ELISA, the ELISA procedure, coupled to a suitable extraction procedure should be subject to peer verification through such avenues as the Association of Official Analytical Chemists (AOAC) Peer-Verified Methods program.

SECTION 4

MATERIALS AND SUPPLIES

ELISA PROCEDURE

Immunochemical Reagents

- Anti-PCB anti-serum, pool AC-3, produced under EPA Contract 68-03-3511.
- Anti-Rabbit IgG-alkaline phosphatase conjugate, Sigma Chemical Company, St Louis, MO, # A-8025.
- Coating Antigen; 4-(2,4,5-trichlorophenoxy)-butyric acid conjugated with bovine serum albumin. Synthesized as described in Section 5.
- Sigma 104 Phosphate substrate tablets, (Sigma Chemical Company, St. Louis, MO.).

Buffers

- Phosphate buffered saline with tween 20 (PBST) pH 7.4; 8.0 g NaCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2g KCl,0.5 mL Tween 20, 0.2 g NaN_3, dissolve and dilute to 1 liter with de-ionized water. Adjust pH as needed with 3N NaOH.
- Carbonate coating buffer, pH 9.6; 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 0.2 g NaN_3, dissolve and dilute to 1 liter. Maximum storage time 2 weeks.
- Substrate buffer; 97 mL diethanolamine, 800 mL H_2O, 0.100 g $MgCl_2 12H_2O$, dissolve and dilute to 1 Liter. Adjust to pH 9.8, store in closed container in dark.

Apparatus/supplies

- Maxisorb I 96-well microplate, or equivalent (A/S NUNC, Roskilde, Denmark).
- Automated plate washer, Skatron Model A/S or equivalent (Skatron, Inc., Lier, Norway).
- Mechanical plate shaker, Bellco Mini-Orbital Shaker, or equivalent (Bellco Biotechnology, Vineland, NJ).
- Microwell plate reader, Molecular Devices Model Vmax, or equivalent (Molecular Devices, Menlo Park, CA).
- Instrument control and data analysis software for the plate reader, Molecular Devices SoftMax, or equivalent (Molecular Devices, Menlo Park, CA).
- Pipettes - repeating pipette capable of delivering 1 mL, adjustable 20 - 200 µL single channel pipette; 0 - 25 µL adjustable positive displacement pipette; 8-channel 50 -

200 µL adjustable pipette; and assorted class A glass volumetric pipettes capable of delivering 15, 20, 25 or 50 mL liquid, dependant on scale of assay.

- Borosilicate glass tubes, 12x75 mm or 13x100 mm.
- Acetate plate sealing tape (Dynatech Laboratories, Chantilly, VA) or equivalent.

EXTRACTION PROCEDURES

Methanol Shake Procedure

- Pesticide grade Methanol
- High density polypropylene wide-mouth screw top bottle, 30-mL, Nalgene 2104-0001 or equivalent (Nalge, Inc., Rochester, NY).
- Stainless steel balls, 1/4" diameter, 5/extraction bottle, (Small Parts, Inc., Miami, FL, # J-BX-4 or equivalent).
- Self-contained syringe filtration unit, 0.45 µM PTFE frit, Whatman Uni-Prep UN113UORG or equivalent (Whatman Laboratories, Clifton, NJ).
- Centrifuge tubes, 15 mL polypropylene.
- Anhydrous sodium sulfate.
- Repeater pipette, capable of delivering 5 mL.
- Mechanical wrist-action shaker, Burrell Model 75 or equivalent (Burrell, Pittsburg, PA).
- IEC Centra-8R centrifuge or equivalent (International Equipment Company, Needham Heights, MA).

Supercritical Fluid Extraction

- Isco Model SFX2-10 Extractor, or equivalent, equipped with a Model 260D Syringe Pump (ISCO, Inc., Lincoln, NE).
- Stainless steel capillary restrictors, 34.5 cm x 50 µm i.d., (ISCO, INC., Lincoln, NE).
- SFE Grade CO_2.
- Anhydrous magnesium sulfate.

Carbon-14 Labeled Tetrachloro Biphenyl Extraction Recovery

- Beckman LS6000IC liquid scintillation counter or equivalent (Beckman Instruments, Inc., Fullerton, CA).

- Ecoscint A scintillation solution or equivalent (National Diagnostics, Manville, NJ).

STANDARDS, REFERENCE MATERIALS AND SPIKING SOLUTIONS

Aroclor Standards

- Aroclors 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268 were purchased as $1x10^{-4}$ g/mL solutions in methanol. (Ultra Scientific, North Kingstown, RI). These primary standards were diluted serially 1:2 into pesticide grade methanol using 10-mL Class A volumetric flasks and 5-mL class A volumetric pipettes, to obtain ELISA standards.

Radiolabeled Tetrachloro Biphenyl

- C-14 Ring labeled 2,2',5,5'-tetrachloro biphenyl, 50 µCi in 55 µL toluene (14.2 Ci/mmol), (Sigma Chemical Company, St. Louis, MO). Diluted to 100 mL in pesticide grade methanol, corresponding to 0.5 µCi/mL or 1.07×10^{-5} g/mL of PCB.

Reference Materials

PCBs in soil standard reference materials (SRMs) were obtained from Environmental Resource Associates, Arvada, Colorado.

- Aroclor 1248
  Two "PCBs in Soil Quality Control Standards" with certified Aroclor 1248 levels of 282 and 33.9 mg/Kg were obtained as mentioned above. The SRMs were prepared by spiking with fresh (unweathered) Aroclor 1248.

- Aroclor 1242
  Five Aroclor 1242 in soil SRMs were obtained as mentioned above. The reference samples were prepared by spiking with unweathered Aroclor 1242 at five concentration levels. The SRMs were spiked at 0.500, 1.50, 8.00, 25, and 100 mg/Kg.

SECTION 5

EXPERIMENTAL PROCEDURES

ELISA PROCEDURE

This protocol is written for one 96-well microplate and should be adjusted accordingly if more samples than can be accommodated on one plate are run.

Day One

Pipette 20 mL carbonate coating buffer into a glass vessel using a 20-mL volumetric pipette. Add 5µL coating antigen (freshly thawed) and mix gently. Transfer 150 µL into each well of the microplate using an 8-channel pipette. Cover plate with acetate plate sealing tape and store overnight at 4 °C.

Pipette 35 mL PBST into a glass flask. Add 11 µL AC-3 anti-PCB serum (freshly thawed) and mix gently. Add 1 mL of this solution to appropriately labeled glass test tubes (12x75 mm or 13x100 mm work well), one tube for each sample, calibration standard or quality control sample. Add 175 µL of the methanol solution containing sample, standard or quality control sample to each tube. Mix gently on a vortex mixer, cap and store overnight at room temperature.

Day Two

Remove sealing tape, place microplate in washer and wash the coating antigen solution from the wells (three times with PBST). Remove microplate from washer, rotate it 180 degrees, and place back into washer. Repeat above wash step. Remove microplate from washer and remove any residual wash solution by inverting plate and rapping it several times on an absorbant paper towel.

Add 100 µL of solution from each of the above tubes prepared during day one to the microplate in triplicate (100 µL/well x three). Cover microplate with plate sealing tape, place it on the mechanical shaker, and shake for three hours at room temperature.

Prepare a solution of the goat anti-rabbit IgG-alkaline phosphatase (GAR-AP) conjugate in PBST to give 15 mL of solution. The dilution factor is lot dependant, but generally ranges between 1:1000 and 1:2500. (The dilution must be experimentally determined prior to assay by running a zero standard using this assay protocol such that an optical density of approximately 1 to 1.5 develops after about 0.5 hr. color development time).

Remove microplate from shaker. Wash and dry as above. Add 100 µL/well of the GAR-AP solution using the 8-channel pipette. Cover microplate with sealing tape and shake gently on the shaker for two hours at room temperature.

Prepare 15 mL of a 1 mg/ml solution of substrate in substrate buffer by placing three substrate tablets into a flask and adding 15 mL substrate buffer with a volumetric pipette.

Remove microplate from shaker, and wash and dry as above. Pay particular care to ensure all the GAR-AP solution and wash solution are removed.

Add 100 µL/well of the substrate solution using an 8-channel pipette. Ensure that the elapsed time between adding solution to the first row of wells and the last row of wells is as short as possible.

Place microplate in the reader and begin reading plate at 405-650 nm at about 15 minutes elapsed time. Continue to make readings until the optical density of the zero standard is about 1.

Perform data reduction as outlined in the SoftMax Documentation. For additional guidance, please refer to "A User's Guide To Environmental Immunochemical Analysis" (Gee et al., 1994).

EXTRACTION PROCEDURES

Methanol Shake Procedure

Five stainless steel balls (cleaned with methanol) are placed into each 30 ml bottle. Five grams of sample is weighed into each bottle. One scoopula tip (approx. two grams) of sodium sulfate is added to each bottle. Five mL methanol is added with the repeater pipette. If the methanol is largely soaked up by a highly absorptive sample, 10 mL methanol can be added and an additional dilution factor is used.

Clamp the bottles into the mechanical shaker, and shake for 20 minutes at maximum amplitude. Remove bottle, and allow sample to settle. Decant the methanol into the base container of the filtration device, filling the container 2/3 full. Insert the capped plunger into the base and push steadily until sufficient filtrate is obtained inside the hollow plunger. Remove cap from the plunger and decant filtrate into appropriately labeled borosilicate screw-top storage vial.

Modification: Some samples, in particular clays, were difficult to filter due to formation of suspensions of very fine particles. The following addition was inserted immediately after removal of bottles from the shaker:

Decant approximately 10 mL of filtrate/suspension into 15-mL centrifuge tube. Cap tube tightly and centrifuge at 2100 g (3900 RPM on the IEC Model Centra 8R) for 35 minutes at room temperature. Remove tubes from centrifuge and decant methanol into the lower container of filtration device. Proceed as above.

Supercritical Fluid Extraction

Add 1 gram anhydrous magnesium sulfate to 4 grams sample in weighing dish. Mix with spatula until all clumps are broken up to obtain a free flowing solid. Transfer samples to extraction vessel, placing two plugs of silanized glass wool over the vessel frits to prevent plugging. Extract at 350 ATM and 100°C for 20 minutes in the dynamic mode. The flow rate of CO_2 was maintained at approximately 1.25 to 1.5 mL/min. using the capillary restrictor heated to 100°C. Collect extract in methanol at ambient temperature. After extraction, adjust volume to 5 mL with methanol.

Soxhlet Extraction Procedure

Follow the extraction procedures in EPA SW-846 Method 3540A (U.S. Environmental Protection Agency, 1990a), substituting pesticide grade methanol for the hexane/acetone solvent

mixture. Remove extract from Soxhlet apparatus. The extracts are ready for ELISA analysis. Do NOT follow the remaining Method 3540A procedures for concentration, cleanup or solvent exchange.

SPIKING PROCEDURES

Method A

Weigh 5 grams of sample into a 25 mL scintillation vial and slowly add pesticide grade methanol until the soil is wetted with a thin film of methanol throughout the sample. Drip the methanol spiking solution containing either the radiolabeled tetrachlorinated biphenyl or Aroclor standard onto the surface of the sample near the center of the container away from the glass walls. Cap the vial and allow to sit overnight. Remove the cap and cover the top of the vial with a kimwipe to prevent contamination of the sample by airborne particulates. Allow the samples to evaporate to dryness at room temperature over several days.

Method B

Weigh 5 grams of sample into a plastic weighing dish and spread out. Slowly drip the spike solutions onto the surface of the samples, taking care to disperse the liquid as widely as possible without allowing the solution to come in contact with the dish. Allow the samples to evaporate to dryness at room temperature.

SCREENING AND CHECKERBOARD TITRATION OF ANTI-SERA AND COATING ANTIGENS

Several objectives were targeted during this phase of development. First, anti-serum which exhibited a high degree of selectivity toward PCBs was identified. Second, the optimal concentrations of the two primary immunochemical reagents (antibody and coating antigen) were determined. Optimal concentrations and activity were determined utilizing a matrix dilution scheme (checkerboard titration) as outlined in "A User's Guide to Environmental Immunochemical Analysis." (Gee, et al., 1994)

Carbon-14 Extract Counting

Methanol soil extracts were added to scintillation solution (400 µL extract/10 mL scintillation solution) and counted using the auto DPM mode according to procedures outlined in the liquid scintillation counter documentation (Beckman Instruments, Fullerton, CA), with a user specified error value of 0.2%.

DEVELOPMENT OF ANTIBODIES FOR PCBs

Immunoassay development requires the production of an antibody specific to the analyte of interest, in this case PCBs. Environmentally significant PCBs are derived from commercially available preparations (Aroclors in the United States) which are mixtures theoretically containing up to 209 congeners. The first consideration which must be addressed in PCB immunoassay development is that of the identity of the immunogen. As a result of trends in production, Aroclors 1242, 1248, 1254, and

1260 are most prevalent in the environment (Hutzinger et al., 1974) and consequently the current assay was targeted to these Aroclors.

PCBs themselves are relatively small immunologically speaking, and are incapable of eliciting the immune response necessary for the generation of anti-PCB antibodies. A general solution to this problem involving small organic molecules entails the synthesis of an analogue molecule possessing a relatively long side chain which itself contains a reactive moiety amenable to further chemical manipulation. This analogue molecule (termed the hapten) is then covalently linked to a large globular protein via the reactive side chain moiety, which by synthetic design, is at or near the end of the side chain. The end result is essentially a large globular molecule with the physico-chemical features unique to the analyte of interest, and large enough to function as an immunogen, that is, to elicit an immune response.

It has been determined that pentachloro biphenyls make up a major fraction of the Aroclors of interest, in fact up to as high as 48% in Aroclor 1254 (Hutzinger et al., 1974). It is expected from chemical principles that the most abundant pentachloro biphenyls in Aroclors are those with one di- and one tri-chlorophenyl ring making up the biphenyl. Nuclear magnetic resonance (NMR) analysis of chromatographically resolved congeners shows this to be the case (Hutzinger et al., 1974). A commonly observed pattern for this pentachloro substitution is the 2,2',4,5,5'-pentachloro biphenyl congener. With this information in mind a hapten based on a 4-hydroxy analog (2,2',4',5,5'-pentachloro-4-biphenylol) was synthesized under EPA Contract 68-03-3511. (U.S. Environmental Protection Agency, 1989). From this analog was prepared a derivative with a functionalized side chain, as shown in Figure 1.

The terminal carboxyl group was then covalently linked with free amino groups on the protein keyhole limpet hemocyanin, to form an amide linked immunogen. Multiple rabbits were immunized with this immunogen, and blood serum collected, resulting in the production of several hundred milliliters of PCB antiserum. Further details can be found in the relevant documents for EPA Contract 68-03-3511.

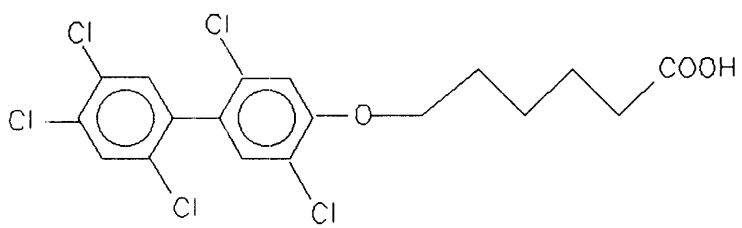

Figure 1. PCB ELISA immunization hapten.

SYNTHESIS OF COATING ANTIGEN

The current PCB immunoassay is based on the "inhibition" ELISA format, as will be described in the next section, and as such, utilizes a PCB analog, or hapten, linked covalently to a carrier protein. The resulting hapten-protein conjugate is termed the coating antigen. For the present assay, 4-(2,4,5-trichlorophenoxy)-butyric acid was chosen as the hapten for the coating antigen due to the analogy with the distal ring of the immunization hapten described in the previous section.

The coating antigen was prepared according to the method of Schmidt (Schmidt, et al., 1990). The hapten was conjugated with bovine serum albumin (BSA) and chicken egg derived conalbumin. Several protein/hapten loading (defined as number of hapten molecules/protein molecule) combinations were synthesized. For the coating antigen incorporated into the quantitative PCB plate ELISA, the hapten loading was estimated spectrophotometrically at 280 nm, and found to be approximately 15. The solution of this coating antigen contained 4.4 mg BSA/mL, based on preparative data.

ASSAY FORMAT

The format employed in the current PCB ELISA is termed an "Inhibition" ELISA. As is the case with all immunoassays, this format relies on the binding of an antibody (Ab) which is specific for a particular analyte (referred to as the antigen in the generic sense). Thus, in the current assay, the antibody for PCBs (the "anti-PCB" antibody) binds specifically with PCB molecules. The details of the indirect inhibition format are depicted in Figure 2 (modified from Gee, et al., 1994).

In the first step, the coating antigen described in section 5 is dissolved in a buffer of basic pH and dispensed into the wells of a polystyrene 96-well microplate. The coating antigen binds with the polystyrene surface by adsorptive forces.

In step 2, samples containing PCBs or PCB calibration standards are added to tubes containing a buffer solution of anti-PCB antibodies. The mixture is allowed to come to equilibrium, at which point the PCB molecules are bound by the antibodies. The quantity of unbound or "free" antibody remaining in solution is dependant on the quantity of PCBs in the sample or standard. Columns designated "A", "B", "C", and "D" in Figure 2 depict varying concentrations of PCBs.

In step 3, the solution from each of the tubes is pipetted into wells on the antigen-coated microplate prepared in step 1 (usually in replicate, for example, 3 wells/tube) and reaction is allowed to proceed to equilibrium, at which point the remaining free anti-PCB antibody binds with the hapten of the coating antigen. The antibodies which bound with PCB in step 2 cannot bind with the coating antigen due to the fact that the binding sites of these antibodies are occupied by PCB molecules. These antibody-PCB bound complexes in solution are then washed away, leaving only antibodies which were able to bind with the coating antigen. Based on this relationship, it is apparent that the quantity of antibodies bound to the coating antigen is inversely proportional to the concentration of PCBs in the sample or standard.

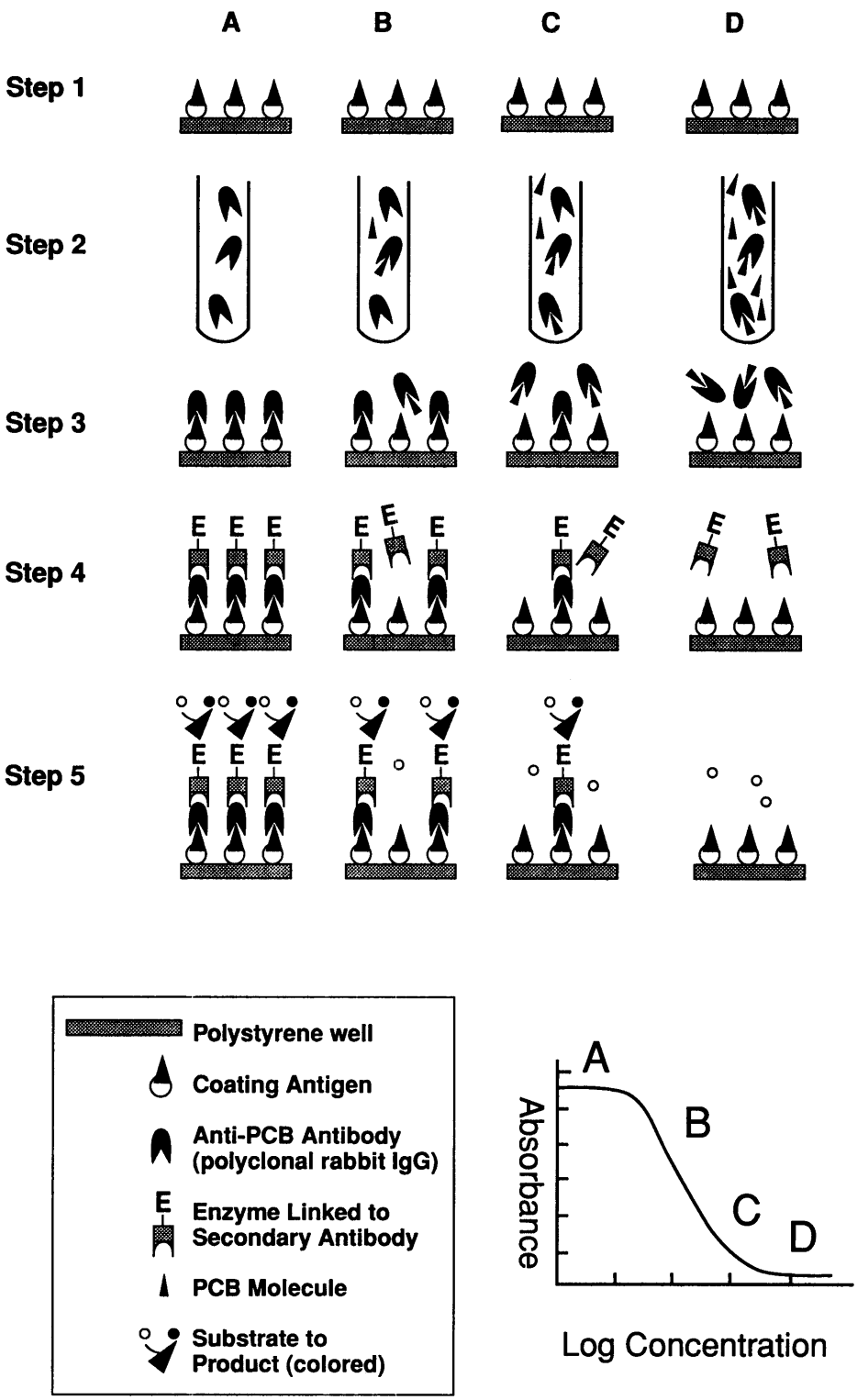

Figure 2. Inhibition ELISA schematic.

In step 4, a second antibody (termed the "secondary antibody") to which an enzyme has been covalently linked is added to each well of the microplate as a solution in buffer. The salient property of this antibody is that it binds with the species specific protein comprising the anti-PCB antibody. For the PCB ELISA, the anti-PCB antibodies were raised in rabbit and are thus composed of rabbit immunoglobulins (rabbit IgG). The secondary antibody, purchased commercially, was raised in goats immunized with rabbit IgG, and is thus an anti-rabbit IgG antibody. The binding reaction is allowed to come to equilibrium, and then the solution is washed away.

Finally, in step 5, a solution of the substrate on which the enzyme acts is added to each well. For the PCB ELISA, the enzyme is alkaline phosphatase, and the substrate is p-nitrophenyl phosphate. The enzyme converts the substrate to a yellow colored product; the more enzyme-antibody conjugate present, the more intense the color development. Due to the inverse relation between PCB concentration and bound primary antibody mentioned in step 2, it also follows that the color formation is inversely proportional to PCB concentration. The assay is completed by measuring the optical density in each well and then mathematically relating concentration of PCB to optical density. This relation then allows for calculation of PCB concentration in the samples based upon their measured optical density values. The response of the ELISA to varying PCB concentrations is plotted in the lower right corner in Figure 2.

Several advantages are gained by using this format. This format typically provides great sensitivity to small changes in concentration, that is, the assay is able to easily discern relatively small differences in concentration between samples. In addition, this is advantageous due to the fact that the enzyme is never exposed to materials that might be present in the sample which might prevent or alter the enzyme-mediated color producing reaction.

SECTION 6

RESULTS AND DISCUSSION

DOSE RESPONSE STUDIES

A characteristic of paramount importance in immunochemically based analytical systems is the response of the system as function of analyte concentration. In the case of ELISAs, this response is physically characterized by measurement of optical density. A typical response function is shown in Figure 3. This response function can be mathematically transformed using a 4 parameter logistic fit of the form:

$$y = (A - D)/(1 + (x/C)^B) + D$$

Where:
- x = Analyte Concentration
- y = Optical Density
- A = Upper asymptote
- B = Slope
- C = Midpoint of Curve
- D = Lower asymptote

This function is plotted in Figure 4.

This curve takes on a characteristic sigmoidal shape. At "low" analyte concentrations, no significant binding has occurred and the response of the system is close to that of a zero concentration standard or sample. As concentration increases, binding proportional to analyte concentration occurs, and the optical density decreases, until finally at "high" analyte concentrations, all of the binding sites on the antibodies are occupied and therefore the system can no longer respond. Of particular significance analytically is the C value; this represents the value at which the system response is at 50%. In immunochemical terminology, it is said that the response is inhibited by 50%, and the C term is thus termed the 50% inhibition level, or the I_{50}. The I_{50} value is a direct indicator of the general concentration level at which the system is capable of functioning.

DOSE RESPONSE FOR COMMON AROCLORS

The current system was characterized for Aroclors 1016, 1221, 1232, 1242, 1248, 1254, 1260 and 1268 using the standard ELISA protocol. Dose response functions for these Aroclors appear in Figures 5-12, and the I_{50} values for each of these Aroclors are summarized in Table 1. Aroclor 1268 showed little response, and hence is not graphically represented.

TABLE 1. FIFTY PERCENT INHIBITION LEVEL FOR AROCLORS

Arolcor	I_{50},ng/mL	Aroclor	I_{50},ng/mL	Aroclor	I_{50},ng/mL
1016	71	1242	25	1260	15
1221	585	1248	22	1262	31
1232	77	1254	10	1268	>500

Interestingly, Aroclor 1254 exhibits the greatest response (lowest I_{50}) of the Aroclors tested, and correspondingly, it is composed of 48% pentachloro biphenyls, the largest percentage of any Aroclor. (Hutzinger et al., 1974).

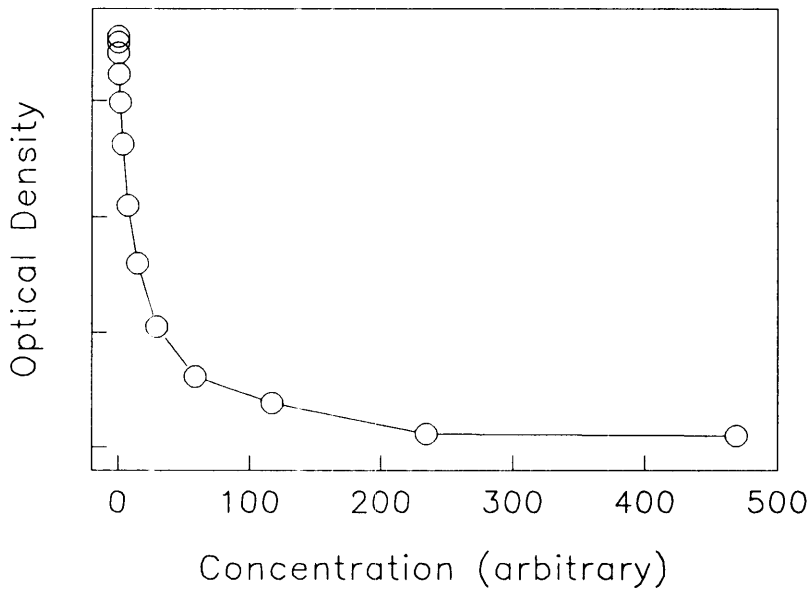

Figure 3. Immunoassay dose response.

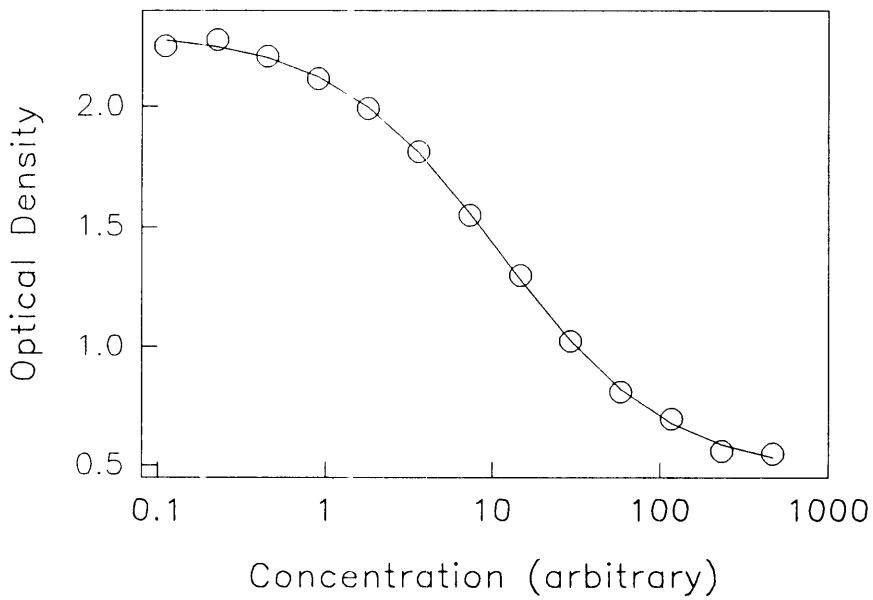

Figure 4. ELISA dose response with four parameter fit.

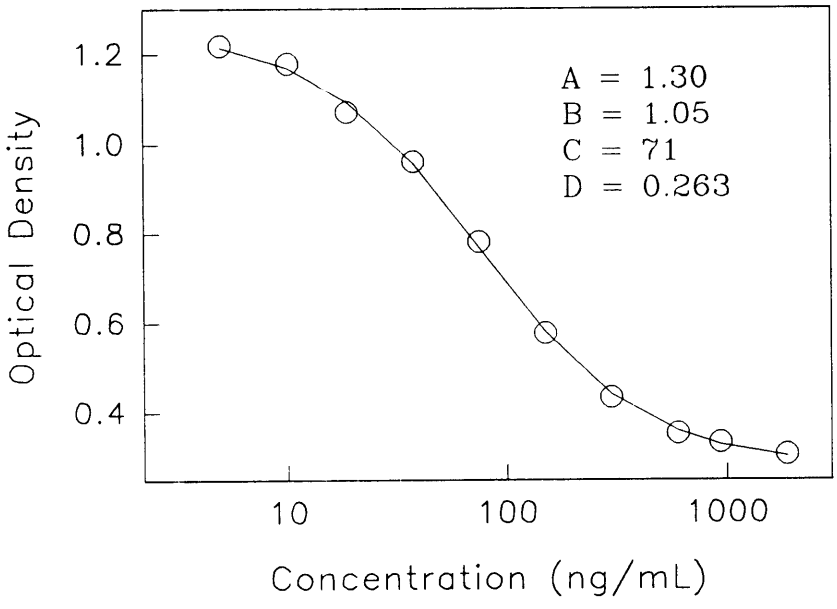

Figure 5. Dose response for Aroclor 1016.

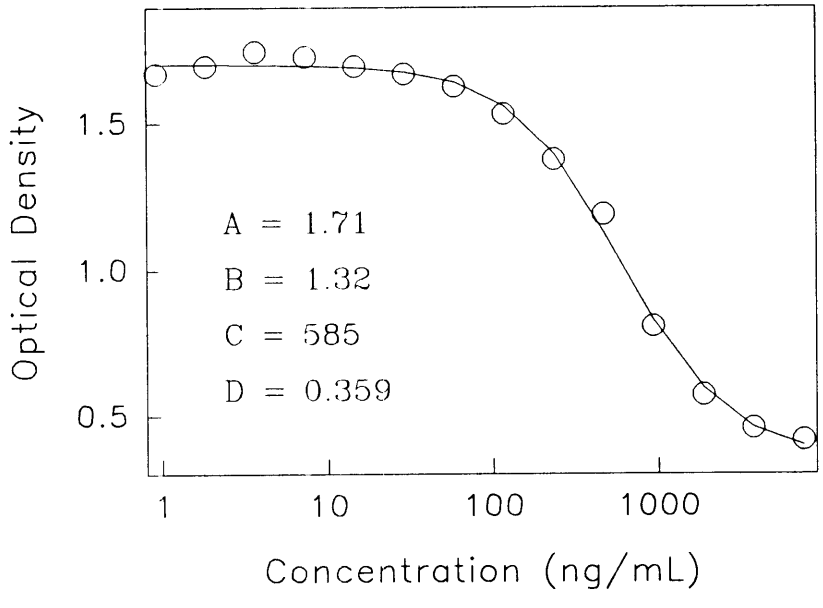

Figure 6. Dose response for Aroclor 1221.

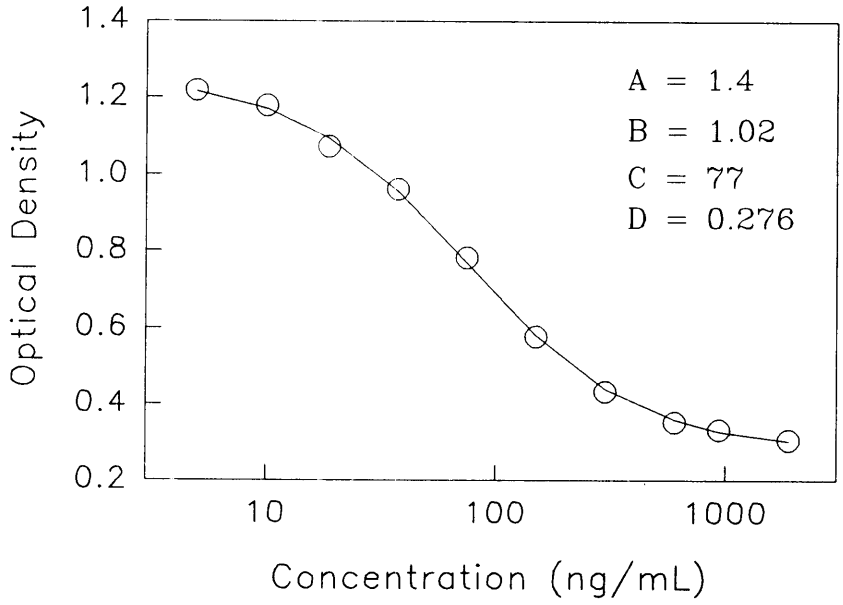

Figure 7. Dose response for Aroclor 1232.

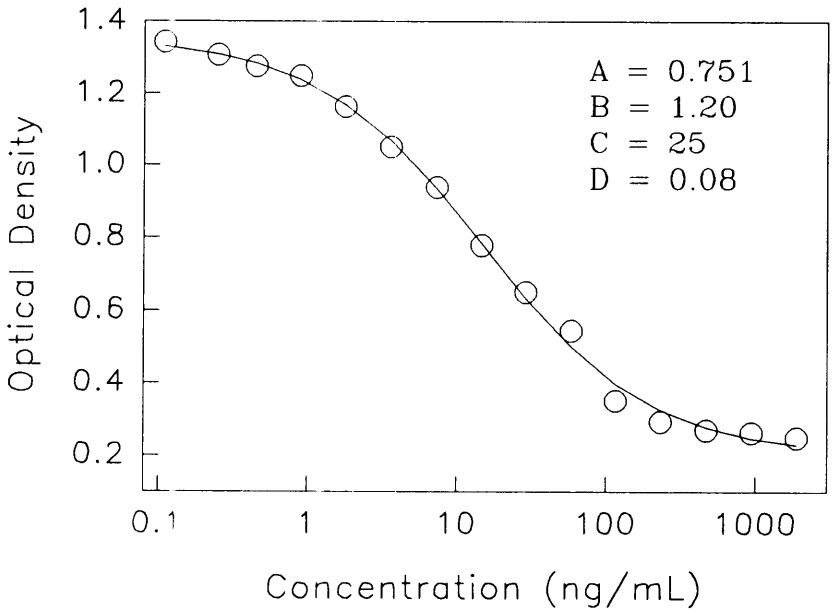

Figure 8. Dose response for Aroclor 1242.

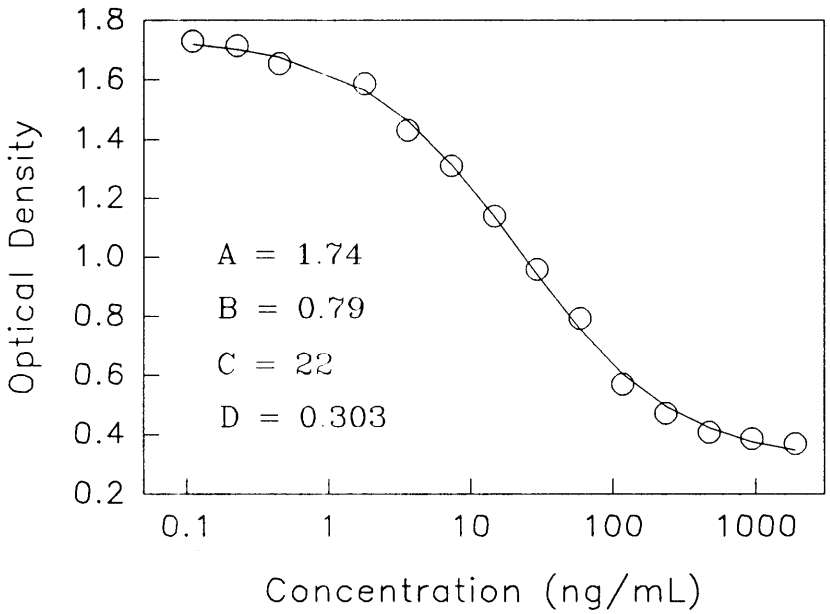

Figure 9. Dose response for Aroclor 1248.

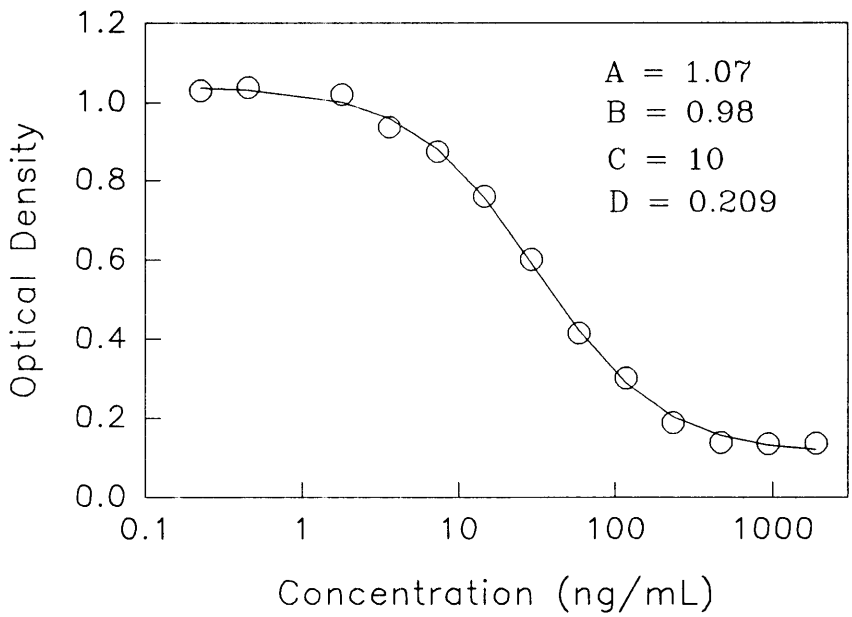

Figure 10. Dose response for Aroclor 1254.

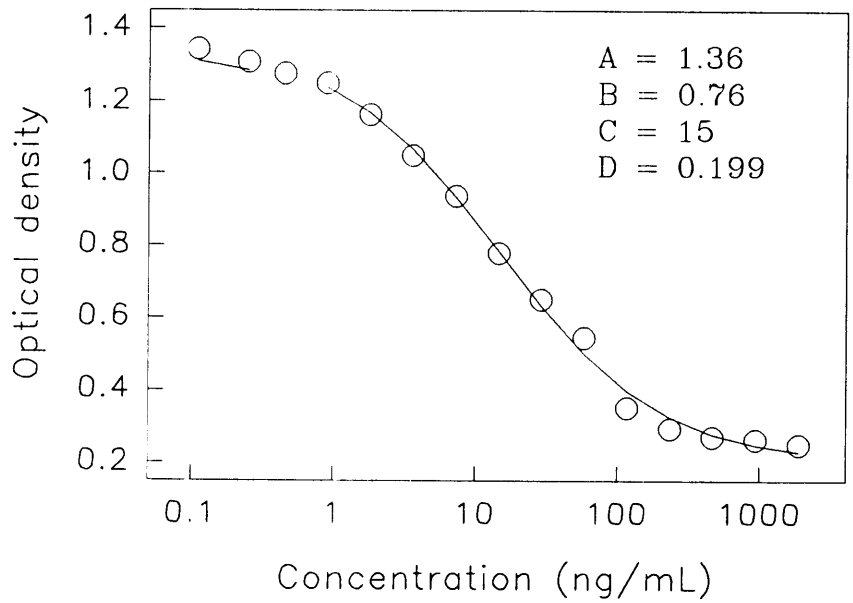

Figure 11. Dose response for Aroclor 1260.

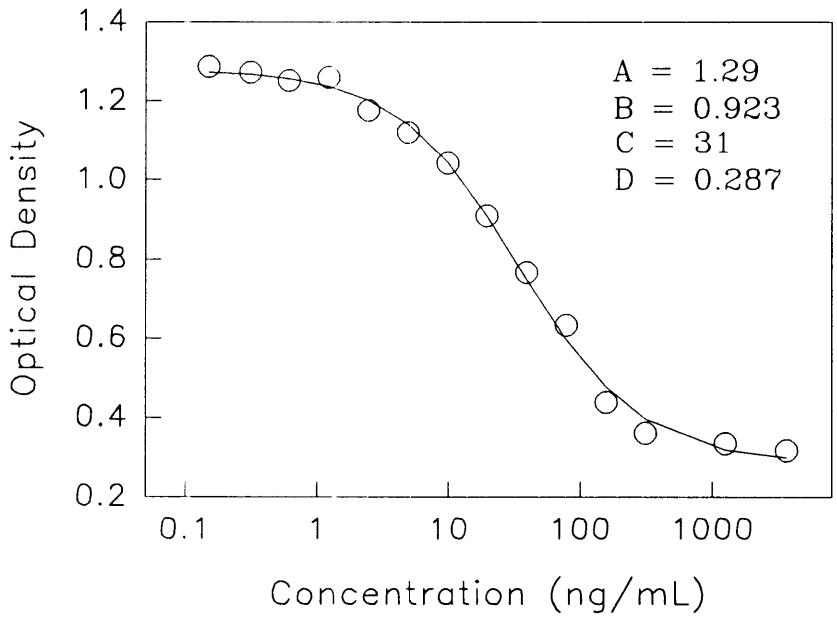

Figure 12. Dose response for Aroclor 1262.

EXTRACTION SOLVENT EFFECT ON ASSAY DOSE RESPONSE

Immunoassay is generally conducted in aqueous solutions which have been buffered. Typically, the immunochemical systems tolerate a high percentage of a number of organic solvents such as dimethyl sulfoxide (DMSO), acetonitrile, 2-propanol, methanol and ethanol. Other solvents, such as hexane, may be tolerated by immunoassays, however in significantly reduced volume fractions. Methanol was chosen as the solvent of choice, based in large part on previous extraction studies employing a range of solvents (Spittler, 1986).

Aroclor 1248 standards, prepared in methanol, were run using the standard assay procedure, varying the concentration of methanol composition in the assay solution from 5, 10, 15 and 20 percent. Dose response curves fit by the 4 parameter logistic fit are illustrated in Figures 13-16. Based on the comparability of curves for the series of 5 to 15 percent methanol, 15 percent methanol was chosen for routine use in the assay, as this will result in the lowest detection limit when sample extracts are added to assay buffer. The dose response for 20% methanol was very erratic, as seen in Figure 16.

CROSS-REACTIVITY STUDIES

Many compounds possibly present in environmental samples may potentially bind, or cross-react, with the anti-PCB antibodies due to some structural similarity with PCBs. The degree of binding will be reduced from that of PCB binding, but nevertheless any cross-reactivity will give rise to a measured level of PCBs higher than that actually present. Of particular interest are 1,2,4-trichlorobenzene and 2,5-dichlorophenol. The trichlorobenzene is analogous to the distal phenyl ring of the immunization hapten, while the dichlorophenol is analogous to the proximal dichlorophenoxy moiety of the immunization hapten. It is also possible that because the hapten is linked to the long aliphatic spacer arm via an phenyl-ether linkage, that chlorophenol ethers might be potentially more cross-reactive than hydroxyl analogues.

To address this issue, serially diluted standard solutions of a number of potentially cross-reacting compounds, based on structural similarity, were prepared in methanol. These solutions were carried through the standard assay procedure, enabling calculation of I_{50} values. These data are summarized in Table 2. In the case when no cross-reactivity was observed at very high concentrations, the I_{50} is reported as greater than the highest concentration of the solution assayed.

The data show that none of the potentially cross reacting compounds exhibits greater than 3% cross-reactivity relative to Aroclor 1248. A 2.7% cross-reaction was observed for 2,4,5-trichloro phenol which is not unexpected considering that the 2,4,5-trichloro substitution pattern was present on the immunizing hapten. This clearly demonstrates the role of hydrogen bonding in this particular cross-reaction, because the corresponding chlorine substituted benzene (1,2,4-trichloro) exhibited insignificant cross reactivity (<0.1% relative to Aroclor 1248).

EXTRACTION EFFICIENCY STUDIES

Extraction of PCBs from soil samples played a significant role in the overall performance of the present ELISA based analytical procedure. In the current study, it was desired that laborious, overnight extraction procedures be avoided. In order to take maximum advantage of the high throughput potential of the quantitative PCB plate ELISA, it was determined that a relatively high

throughput extraction procedure should be employed, and thus the extraction procedure was derived from known procedures used for field applications (U.S. Environmental Protection Agency 1992a, 1992b).

Generally, these extraction methods entail placing the soil sample, drying agent and extraction solvent in a suitable vessel, followed by hand shaking for several minutes. The method derived from these procedures and used in the current study is more vigorous, in that it employed a mechanical shaker of much greater intensity than obtained by handshaking, and a longer time period.

The question of determining extraction efficiency of a method as applied to real-world samples is never directly answerable unless one has an infinite amount of time and resources to enable preparation of spiked samples under the same real-world conditions as those which produced the samples in the first place. As a substitute for this impractical situation, a three pronged approach was taken in the current study.

The first part of the approach involved spiking of commercially obtained PCB standard reference soils (SRMs) and real-world samples with known quantities of radiolabeled analyte, followed by sample aging and then subsequent extraction and counting of the extracted radioactivity. Carbon-14 ring labeled 2,2',5,5'-tetrachloro biphenyl was chosen as the spiking material. In the case of PCBs, this does not strictly emulate extraction of real world Aroclor contaminated soils, due to the fact that

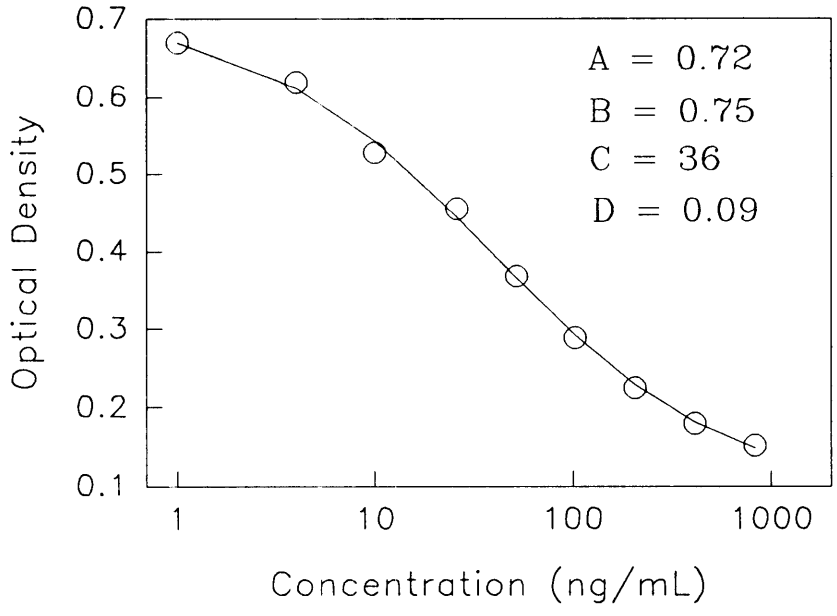

Figure 13. Dose response, 5% methanol.

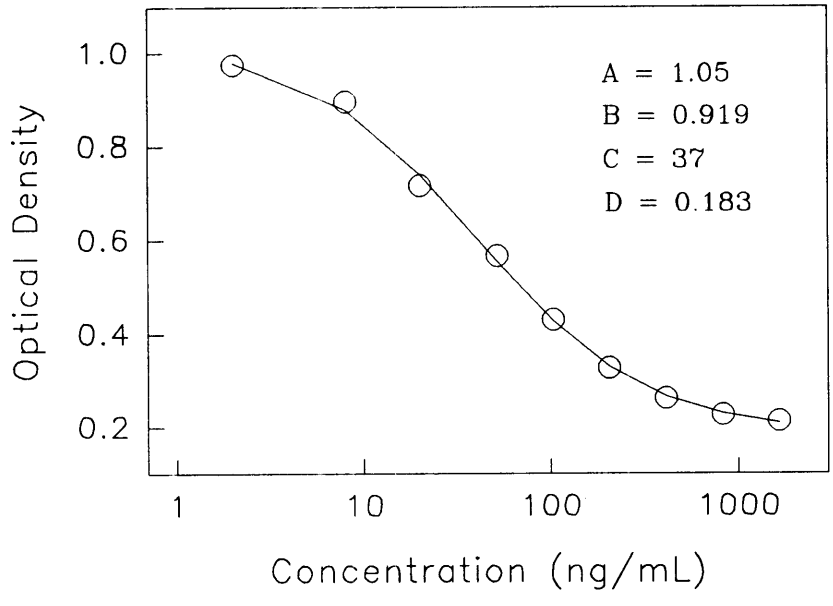

Figure 14. Dose response, 10% methanol.

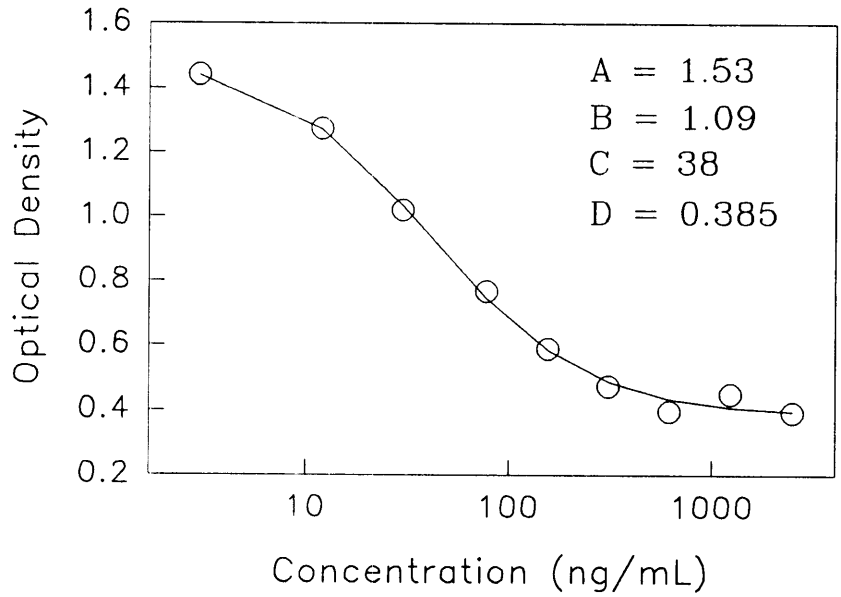

Figure 15. Dose response, 15% methanol.

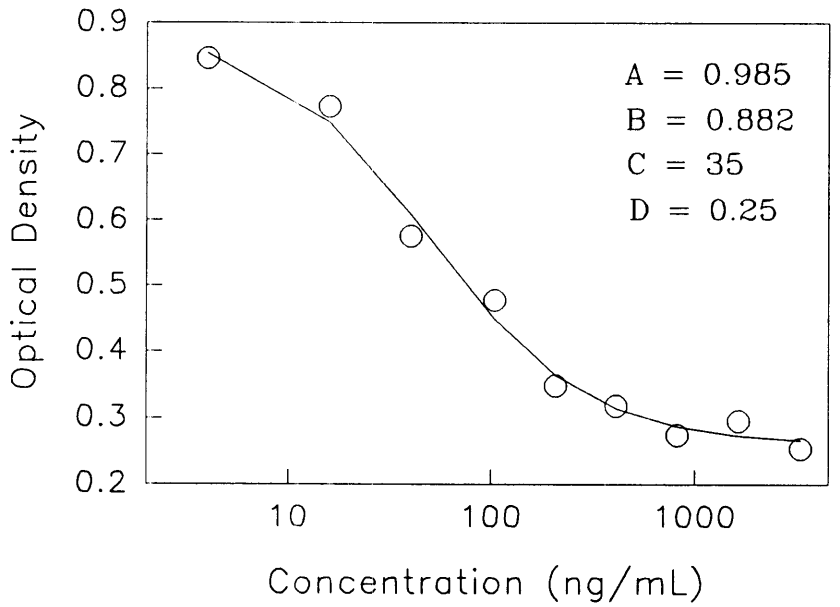

Figure 16. Dose response, 20% methanol.

TABLE 2. CROSS-REACTIVITY OF POSSIBLE CO-CONTAMINANTS

COMPOUND	I_{50}, ng/mL
Anisole, 2,3,4-trichloro	>20000
Anisole, 2,4,6-trichloro	>20000
Anisole, 2,6-dichloro	>20000
Anisole, 2-chloro	>20000
Anisole, 3,5-dichloro	>20000
Anisole, 4-chloro	>20000
Benzene, 1,2,3,4-tetrachloro	>20000
Benzene, 1,2,3-trichloro	>20000
Benzene, 1,2,4,5-tetrachloro	8920
Benzene, 1,2,4-trichloro	>20000
Benzene, 1,2-dichloro	>20000
Benzene, 1,3,5-trichloro	>20000
Benzene, 1,3-dichloro	>20000
Benzene, 1,4-dichloro	>20000
Benzene, chloro	>20000
Biphenyl	>20000
Butyric acid, 4-(2,4,5-trichlorophenoxy)	5300
DDE	>1400000
DDT	>205500
Phenol, 2,3,4-trichloro	3940000
Phenol, 2,3,5,6-tetrachloro	1280
Phenol, 2,3,5-trichloro	>20000
Phenol, 2,3,6-trichloro	>20000
Phenol, 2,3-dichloro	29300
Phenol, 2,4,5-trichloro	914
Phenol, 2,4,6-trichloro	>20000
Phenol, 2,4-dichloro	7990

COMPOUND	I_{50}, ng/mL
Phenol, 2,5-dichloro	8740
Phenol, 2,6-dichloro	81900
Phenol, 2,6-dichloro	>20000
Phenol, 2-chloro	>20000
Phenol, 3,4-dichloro	98000
Phenol, 3,5-dichloro	1290
Phenol, 4-chloro	>20000
Phenol, pentachloro	>20000

Aroclors are of course mixtures of many congeners. Unfortunately, radiolabeled Aroclors are not commercially available. In order to use radiolabeled Aroclors, it would be necessary to chlorinate radiolabeled biphenyl under conditions which essentially emulated the industrial preparation of the Aroclors. This was clearly undesirable in terms of the additional research effort needed. The tetrachloro congener named above was chosen as the closest approximation from the limited list of commercially available radiolabeled congeners because it has been determined that this congener typically comprises up to 8.4% in Aroclor 1242 and 1248 (Albro et al., 1981).

The second approach by which method extraction efficiency was ascertained was by extraction of commercially available PCBs in soil standard reference materials (SRMs), followed by analysis of the extracts by the quantitative PCB plate ELISA. This approach may potentially be limited by the fact that the data is confounded by ELISA performance and by the fact that the soil matrix may not necessarily emulate the actual real-world samples. In the current study, two Aroclor 1248 SRMs representing 2 PCB levels were extracted. In addition, five Aroclor 1242 SRMs representing 5 PCB levels were extracted.

The third approach to determining extraction efficiency involved spiking of approximately 10 percent of the real world samples with known quantities of the relevant Aroclor, followed by extraction and subsequent analysis by the ELISA.

EXTRACTION OF C-14 RADIOLABELED TETRACHLORO BIPHENYL SPIKED SOILS

Samples

Four separate sets of extraction experiments were conducted aimed at examining the effect of PCB level on the extraction efficiency, the effect of changing the spike level, and the effect of different soil types on the extraction efficiency. All spikings were carried out employing spiking Method A.

Extraction of USATHEMA Soil

The first set of extractions utilized clean standard soil obtained from the United States Army Toxic and Hazardous Environmental Material Agency (USATHEMA). A total of thirty 5-gram samples were spiked; 10 of these samples were spiked with 58 µL of C-14 spiking solution to give a total PCB concentration of about 0.1 mg/Kg. Twenty 5-gram samples were spiked with 125 µL of C-14 spiking solution and half were fortified with 32 µL of 1.1 X 10^{-5} g/mL of Aroclor 1248 standard solution, to give a total PCB level of 1 mg/Kg. The other half were fortified with 454 µL of the 1248 standard solution to give a total PCB level of 10 mg/Kg. In addition, three unspiked blanks were carried through the procedure.

The samples were extracted with 5 mL methanol according to the described procedure. The spiking vials were also extracted with 5 mL methanol to determine if any losses of activity occurred during the spiking procedure. The extract was added to liquid scintillation cocktail (400 µL extract/10 mL cocktail), and the samples were counted. In addition, calibration standards were prepared by adding 125 µL of the C-14 spiking solution to 5, 7, 10, and 15 mL of methanol. The standards were added to scintillation cocktail (same as above, in triplicate); these standards correspond to 100, 71, 50 and 33 percent recovery for the 1 and 10 mg/Kg samples and 216, 153, 108 and 72 percent recovery for the 0.1 mg/Kg spikes. The standards were counted along with the spiked samples, and a linear least-squares fit was used to calculate percent recovery. The fit is shown in Figure 17. The results are presented in Table 3.

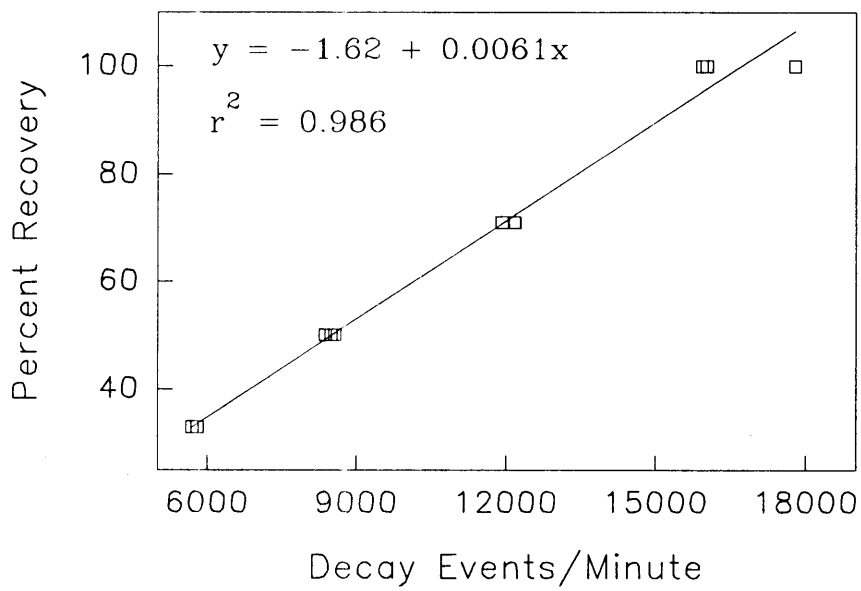

Figure 17. USATHEMA C-14 extraction calibration curve.

TABLE 3. RECOVERY OF RADIOLABELED TETRACHLORO BIPHENYL FROM USATHEMA SOIL

Total PCB Level, mg/Kg	Mean Percent Recovery of C-14	RSD, %
0.1	84.7	0.2
1.0	89.1	1.5
10	89.9	0.6

No significant activity was counted in the spike vials, thus the calculated recoveries represent actual recoveries of material from the soil. This data demonstrates that the C-14 labeled tetrachloro biphenyl can be recovered with high efficiency in the presence of up to 10 mg/Kg Aroclors.

Extraction of Commercial PCB Reference Soils

The second set of C-14 extractions was carried out on seven commercially obtained PCB in soil SRMs (Environmental Research Associates, Arvada, CO). Five levels of Aroclor 1242 were represented, along with two levels of Aroclor 1248. The samples were weighed out in duplicate, and were prepared, extracted and analyzed using the described procedures. The calibration curve for this extraction is shown in Figure 18. The results are presented in Table 4.

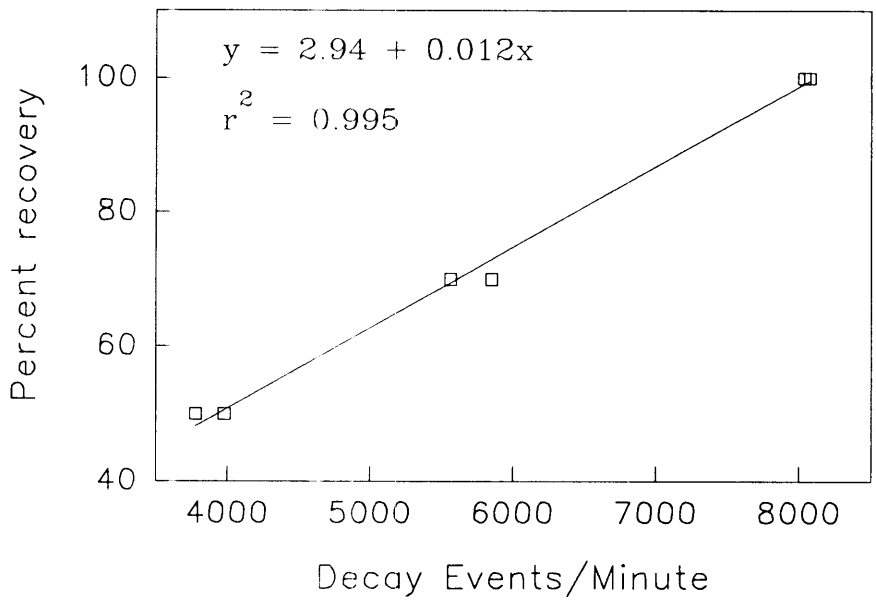

Figure 18. Reference Soil C-14 extraction calibration curve.

TABLE 4. RECOVERY OF RADIOLABELED TETRACHLORO BIPHENYL FROM SOIL SRMS

Aroclor, SRM Concentration (mg/Kg)	Percent C-14 Recovery
1248, 33.9	87.6
1248, 33.9	94.5
1248, 282	87.0
1248, 282	96.0
1242, 0.5	84.0
1242, 0.5	90.8
1242, 1.5	88.5
1242, 1.5	86.0
1242, 8.0	94.0
1242, 8.0	89.0
1242, 25.0	91.0
1242, 25.0	90.0
1242, 45.0	88.0
1242, 45.0	88.0

The mean extraction efficiency of the C-14 radiolabeled PCB in the presence of widely varying PCB levels and two types of Aroclors is 89.4%, with a RSD of 3.7%. Again, no significant activity remained on the spiking vessel walls, thus the calculated recoveries represent about an 11% residual non-recovery in the soil samples.

Extraction of C-14 Spiked Real-world Samples

The third set of extractions was carried out on approximately 10% of one of the sets of real-world samples included in the present report. These samples were collected at Kansas City, MO, and have known analytical values for Aroclor 1248 contamination levels, as to be discussed later. Spiking, extraction, and analysis were carried out in duplicate, identical to procedures described above, with the exception that the samples were dried and powdered prior to spiking due to the difficulties encountered in spiking firm clay samples. The results for the extractions are given in Table 5.

TABLE 5. RECOVERY OF RADIOLABELED TETRACHLORO BIPHENYL FROM KANSAS CITY SOIL/CLAY SAMPLES

Sample ID	Percent C-14 Recovery	Aroclor 1248 level, mg/Kg
KC043D	88.7	1.74
KC043D	94.0	1.74
KC046	87.9	<0.033
KC046	88.4	<0.033
KC049	87.4	<0.033
KC049	87.4	<0.033
KC061	90.8	580
KC061	97.5	580
KC068	90.7	0.504
KC068	96.9	0.504
KC081	89.8	0.687
KC81B	92.7	0.687
KC083D	89.2	0.413
KC083D	94.6	0.413
KC085	98.9	428
KC085	98.1	428
KC088	89.0	2.70
KC088	93.7	2.70

The mean recovery was found to be 92.0%, with a RSD of 4.1%. The results indicate that the extraction procedure can remove with a greater than 90% efficiency a major congener of the Aroclors of interest in the current study, and further, can do so regardless of level of Aroclor 1248 present.

ELISA PERFORMANCE CHARACTERISTICS

The quantitative PCB plate ELISA performance was characterized in terms of detection limit, assay accuracy, assay precision and the practical quantitation range.

Assay Detection Limit

The assay detection limit is defined as being three standard deviations above the zero standard. Optical density data for twelve separate determinations spanning an approximately 5.5 month period were used in the calculation. The standard deviation for the zero standard (triplicate wells) was multiplied by three and subtracted from the optical density of the zero standard (recall the inverse relation between concentration and optical density). The resulting optical density value was substituted into the respective four-parameter fit for each dose response curve.

The resultant concentration values of Aroclor 1248 corresponding to the assay detection limit are summarized in Table 6:

TABLE 6. ELISA DETECTION LIMIT FOR AROCLOR 1248

Calculated Detection Limit, ng/mL
0.742
0.336
1.56
0.608
2.80
0.767
2.34
1.55
1.40
2.90
0.646
0.527
Mean Detection Limit ± 1 Standard Deviation, ng/mL = 1.34 ± 0.87

Similar data were acquired for Aroclor 1242 using identical procedures. These data are summarized in Table 7.

TABLE 7. ELISA DETECTION LIMIT FOR AROCLOR 1242

Calculated Assay Detection Limit, ng/mL
1.62
1.56
0.608
1.83
0.776
2.33
2.32
Mean Detection Limit ± 1 Standard Deviation = 1.57 ng/mL ± 0.62 ng/mL

It is important to note that these are not detection limits for soil samples, but rather for concentrations in the assay solution. When converted to corresponding soil levels, these values are corrected for the dilution factor of 6.67 which occurs when adding 15% soil extract to 85% buffer. Hence the limit of detection for 1248, in soil, is 8.95 ng/g ± 5.8 ng/g. The calculated detection limit for Aroclor 1242 in soil is 10.5 ng/g ± 4.1 ng/mL. The calculated standard deviations are fairly large fractions of the calculated detection limits, which in large part is due to the fact that calculations of concentrations within in the region near the zero standard are inherently of low mathematical accuracy when the slope of the four parameter fit approaches zero.

Standard Curve Characteristics

The practical quantitation range for the ELISA corresponds to the linear region of the sigmoidal curve where the optical density begins to fall off rapidly as PCB concentration increases. This region can be conveniently fit mathematically by a linear equation of the form:

$$\mathbf{OD = A + B*log(Concentration)}$$

Where:

OD = measured optical density
A = intercept value
B = slope of the line
C = Concentration of PCBs in ng/mL

A typical calibration curve is shown in Figure 19. Data points are the mean of triplicate measurements with error bars of $\pm$ 2 standard deviations.

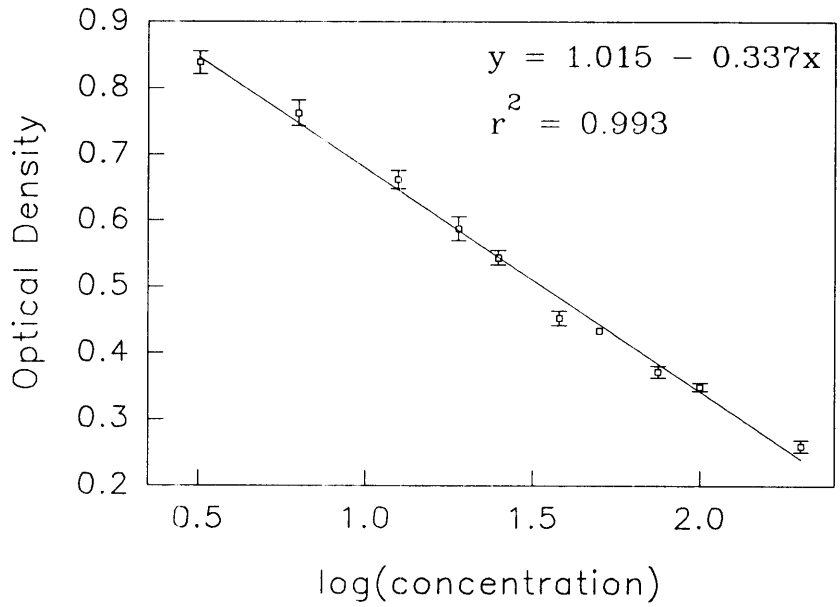

Figure 19. Optical density versus log Aroclor 1248 concentration.

As can be seen, the correlation coefficient, r^2, is greater than 0.99; the upper and lower limits for quantitation can be empirically reached by including standards which typically fall in the high and low regions of the four parameter curve where the response begins to roll off. Calibration curves with r^2 values less than 0.99 were never used for calculation of Aroclor concentration in samples. Rather, the points at the end of the curve were discarded, resulting in appropriate inclusion of the linear region.

The current assay has been employed in the range of about 3 ng/mL to 200 ng/mL for Aroclors 1248 and 1242 in assay solution, corresponding to 20 to 1333 ng/g in soil. These concentrations represented the maximum assay working range, as slight roll-off was occasionally observed for these boundary concentrations as a result of inter-assay variation. Typically, inclusion of these points (if rolling off) would lower the correlation coefficient into the 0.97 region, in which case these boundary points were discarded.

Long Term Assay Precision

To address the question of assay stability and reproducibility, standard reference materials (SRMs) were assayed during each analytical run. For Aroclor 1248, two commercially obtained soil SRMs (Environmental Resource Associates, Arvada, CO) were used, with certified Aroclor 1248 levels of 33.9 and 282 mg/Kg of soil. For Aroclor 1242, five commercially available soil SRMs (Environmental Resource Associates, Arvada, CO) were used, with certified Aroclor 1242 levels of 0.5, 1.5, 8, 25 and 100 mg/Kg of soil.

In all cases, the soil SRMs were extracted employing the standard extraction procedure as described, and the extracts stored in borosilicate glass vials sealed with teflon lined caps. The extracts were diluted with methanol to bring the PCB levels into the assay range.

Long Term Assay Precision for Aroclor 1248--

The Aroclor 1248 SRMs were extracted in duplicate and are designated as "A" and "B." These extracts were assayed over a 6 month period, and thus, the resulting data represent inter-assay variability for a six month period. No apparent trends, either upward or downward, were noted as a result of storage artifacts. The post-extraction dilution for the Aroclor 1248 SRMs corresponded to a dilution factor of 334 (before being added to assay) for the low level SRM and a dilution factor of 2263 (before addition to assay) for the high level 1248 SRM. Results for the Aroclor 1248 SRMs are summarized in Table 8.

The optical densities for the diluted Aroclor 1248 SRMs corresponded to optical densities in the range corresponding to the region between the 38 and 50 ng/mL calibration standards, thus these sample dilutions fell into a mathematically optimal region, i.e., the central part of the curve. The reported error does not include an estimate of extraction or dilution error.

Long Term Assay Precision for Aroclor 1242--

The five Aroclor 1242 SRMs were extracted on two separate occasions and analyzed by ELISA. It was observed that the results for the first set of extracts did not differ from those of the second set of extract. Due to the large number of wells needed to assay two sets of five standards (three wells/sample x five x two sets = 30), it was decided that one set/analysis was the practical limit which still enabled analysis of a reasonable quantity of samples on a plate. The stored extracts were analyzed over a 3 month period, to give Aroclor 1242 SRM data representing 3 month variation data, as summarized in Table 9.

The measured levels of Aroclor 1242 for all soil SRMs are on the low side, corresponding to about 53-91% extraction efficiency, assuming 100% accuracy for the ELISA. According to SW-846 Method 8080, a measured recovery of 39-150% for solution phase Aroclor 1242 measurements meets quality control criteria for acceptable results. The quoted Method 8080 recovery criteria does not take into account the additional error incurred as a result of soil extraction, thus, it can be concluded that the ELISA results represent acceptable performance, at least based on comparability to gas chromatographic reference data.

Based on data which will be discussed in the sections addressing spike recovery and matrix effects, it can be concluded that the ELISA itself is accurate, and thus it can be stated that the low results are most likely due to low extraction efficiency for the Aroclor 1242 SRMs.

EFFECT OF SAMPLE MATRIX ON ASSAY PERFORMANCE

It is imperative to determine what effect, if any, non-analyte related sample characteristics had on assay performance. In the case of immunoassay, such factors as ionic strength, metal and other inorganic content, humic acid content, etc., may result in an alteration of the assay performance. The effect of matrix upon assay performance, when observed, is of a non-specific nature. By this, it is meant that the matrix effect is not due to binding events with the antibody itself, as is the case with analyte or a cross-reacting compound, but rather with such ill-characterized effects such as alteration of antibody conformation. This latter event could alter the binding constant for the antibody-analyte interaction.

If any such effects were to occur, there would be a loss of correspondence between the dose response for a set of "clean" standard solutions, and a set of "matrix effect containing" samples. As a

result, the accuracy and possibly precision of the analytical results could differ greatly from the characterized accuracy and precision of the assay system.

An effective method by which to detect such matrix effects is to carry out a series of dilutions on the samples, assay these sample diluates, and then determine mathematically whether the results are related. Such a scheme is termed a determination of parallelism. If the analytical results for the diluates fall within the working range of the PCB assay, two conclusions can be drawn about the measured PCB values.

First, the quantity of PCB measured for all the diluates from a given sample should be identical within the bounds of method accuracy after mathematical correction for dilution. Second, it is clear that with respect to PCB concentration, the serial dilutions for the samples are essentially equivalent to the serial dilutions used to prepare the calibration standards, and as such, the sample diluates ought to produce a dose response or calibration curve mathematically identical to that of the standard curve obtained during the same analytical run. If this is not the case, it becomes apparent that other matrix related effects are skewing the assay response.

Importantly, it should be noted that such a scheme cannot determine whether cross-reacting compounds are contributing to the measured apparent PCB concentration, because the cross-reactivity is an equilibrium binding event just as is the PCB-antibody binding event. Essentially, the two events differ only in the magnitude of their respective binding constants.

Parallelism Determination Results

Parallelism studies were carried out on each of the three sets of real world samples. Serial dilutions were performed by adding 1 mL of the methanolic sample extract to 1 mL of pesticide grade methanol. Following mixing, 1 mL of this diluate was added to another mL of pesticide grade methanol and then mixed. This procedure was repeated until dilutions which gave results within the working range of the assay were obtained. The resulting diluates had dilution factors of 2^n, where n is any integer between 1 and up to 8. The results are summarized in two ways. The results for all diluates of a given sample falling within the assay working range were averaged, and a RSD calculated. In addition, the data are plotted superimposed onto the assay dose response curves for the standard solutions.

TABLE 8. ELISA RESULTS FOR AROCLOR 1248 SOIL SRM EXTRACTS

High A (mg/Kg)	High B (mg/Kg)	Low A (mg/Kg)	Low B (mg/Kg)
258	238	37.8	35.0
275	247	39.6	35.3
296	288	33.5	-----
284	273	34.6	31.8
261	244	40.6	33.5
299	287	37.9	33.0
291	287	39.2	33.6
242	248	33.0	29.2
219	218	26.6	26.9
313	291	39.3	39.6
295	286	36.7	37.4
316	285	44.4	39.9
263	245	39.8	33.4
239	231	33.7	31.2
302	284	36.2	32.3
245	237	34.6	32.0
264	256	38.4	37.2
247	236	33.2	30.4
273	274	37.2	38.6
Expected: 282	Expected: 282	Expected: 33.9	Expected: 33.9
Mean = 273	Mean = 261	Mean = 36.6	Mean = 33.9
Percent Error = -3.2	Percent Error = -7.4	Percent Error = 7.9	Percent Error = 0
SD = 26.6	SD = 23.4	SD = 3.76	SD = 3.49
RSD = 9.8%	RSD = 8.8%	RSD = 10.3%	RSD = 10.2%

TABLE 9. ELISA RESULTS FOR AROCLOR 1242 SOIL SRMS

ERA 0.5 mg/Kg	ERA 1.5 mg/Kg	ERA 8 mg/Kg	ERA 25 mg/Kg	ERA 100 mg/Kg
0.258	0.940	5.93	17.7	99.6
0.283	0.879	5.44	16.7	91.3
0.179	0.718	4.64	14.8	94.3
0.205	0.801	5.07	16.8	91.4
0.230	0.820	4.96	15.9	87.3
0.309	0.904	4.53	16.2	-----
0.361	0.929	6.70	16.6	-----
0.357	0.980	7.67	19.1	-----
0.301	0.779	3.67	17.7	-----
0.179	0.756	4.97	16.9	-----
0.283	0.879	5.44	16.7	91.3
0.226	1.261	7.00	18.8	88.9
0.237	0.988	5.96	18.3	94.1
0.329	0.911	6.67	18.7	83.3
0.226	0.751	4.41	-----	-----
0.254	0.946	6.23	17.5	96.7
0.266	0.916	5.72	17.1	87.6
Expected: 0.500	Expected: 1.50	Expected: 8.00	Expected: 25.0	Expected: 100
Mean = 0.264	Mean = 0.892	Mean = 5.22	Mean = 17.221	Mean = 91.444
Percent Error = -47	Percent Error = -40.5	Percent Error = -35	Percent Error = -31	Percent Error= -8.6
SD = 0.054	SD = 0.122	SD = 1.65	SD = 1.11	SD = 4.41
RSD = 20.0%	RSD = 13.7%	RSD = 31.0%	RSD = 6.5%	RSD = 4.8%

Parallelism Results for Aroclor 1248 From the Kansas City Samples--

Approximately ten percent of the Kansas City SITE samples were non-randomly selected based on having concentration ranges between 818 and 2498 µg/Kg, as previously determined by ELISA. These samples were selected because the concentrations were such that several of the serial dilutions starting directly from the undiluted sample extracts would have concentrations falling within the assay working range. If higher concentration samples had been chosen, the number of serial dilutions needed to bring the concentrations into the assay working range would be so high such that the matrix effect, if any, would have already been diluted out. Initial concentration below 800 µg/Kg were not suitable because, once diluted several times, the concentrations would quickly fall off the lower end of the assay working range.

The results for the calculated concentrations are summarized in Table 10.

TABLE 10. ELISA RESULTS FOR SERIALLY DILUTED KANSAS CITY SOIL EXTRACTS

Sample	Mean PCB Concentration (µg/Kg)	RSD (%)	Dilution Range Measured
KC17	2798	4.4^{B}	$2^{2}..2^{4}$
KC22D	406	13.8^{C}	$2^{1}..2^{4}$
KC39	968	14.0^{C}	$2^{1}..2^{4}$
KC42	1514	0.9^{B}	$2^{2}..2^{4}$
KC53	1707	11.7^{C}	$2^{1}..2^{4}$
KC56	924	16^{A}	$2^{1}..2^{2}$
KC60D	1590	11.9^{B}	$2^{1}..2^{3}$
KC68	1252	7.1^{C}	$2^{1}..2^{4}$
KC83	777	8.8^{C}	$2^{1}..2^{4}$

A: n = 2, B: n = 3, C: n = 4

The average RSD for all samples (excluding KC56, for which only two points fell within the assay working range) is 9.0%. This is within the precision performance of the assay for repeated samples at a fixed concentration. It can be concluded that, based on the above results, no significant matrix effect was observed in the Kansas City samples. The data for the Kansas City diluates are plotted in Figures 20-22. Each plot represents data collected during one analytical run. The data for samples is plotted along with the calibration standards used in the corresponding run.

The assay response for serially diluted sample extracts is essentially identical to assay response for calibration standards, thus it can be concluded that no matrix effects were present.

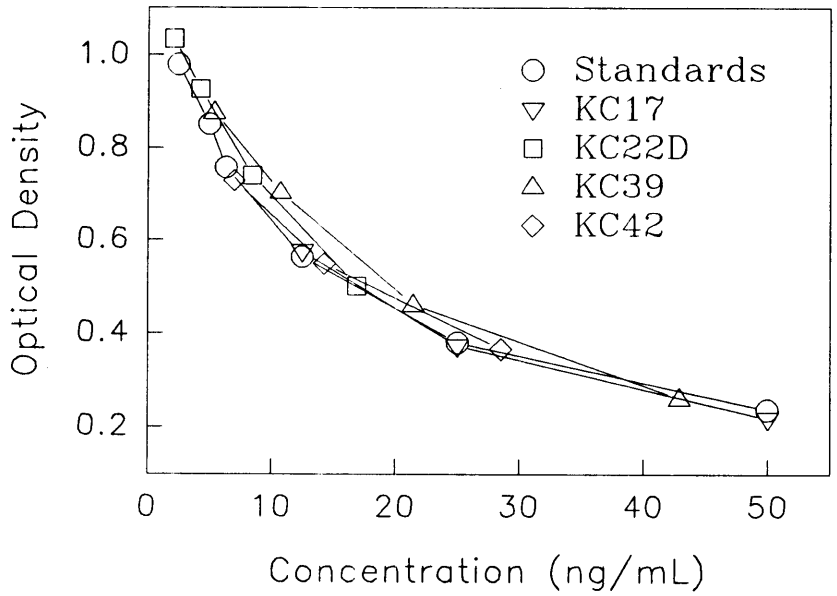

Figure 20. Dose response for serially diluted Kansas City samples, group 1.

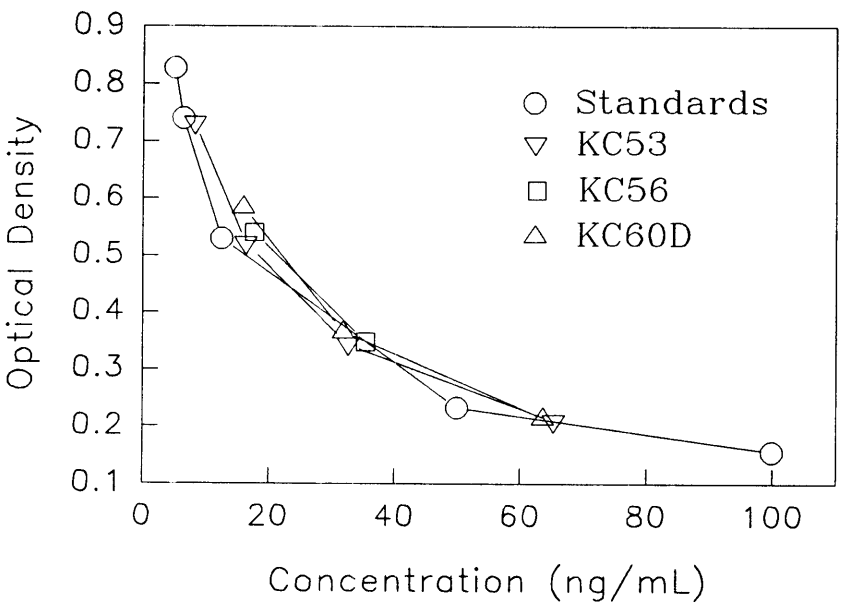

Figure 21. Dose response for serially diluted Kansas City samples, group 2.

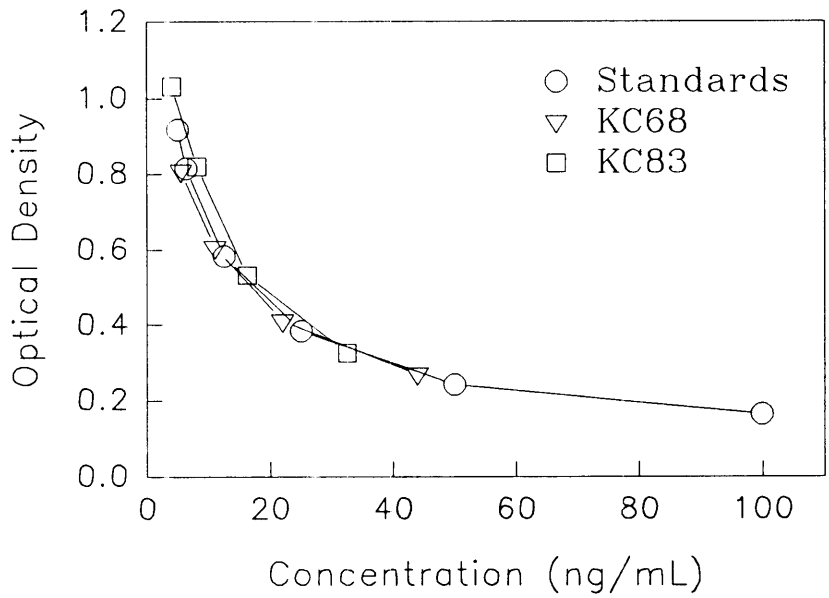

Figure 22. Dose response for serially diluted Kansas City samples, group 3.

Parallelism Results for Aroclor 1242 From the Allied Paper/Portage Creek/Kalamazoo River Samples--

Eleven samples from the Allied Paper/Portage Creek/Kalamazoo River Superfund site were serially diluted and subsequently the diluates were analyzed by ELISA as described. The results are summarized in Table 11.

TABLE 11. ELISA RESULTS FOR SERIALLY DILUTED ALLIED PAPER SAMPLES

Sample	Mean Aroclor 1242 Concentration, (mg/Kg)	RSD (%)	Dilution Range
19A	98.3	9.4[B]	2^2-2^4
24B	130	8.4[C]	2^2-2^5
20B	74.4	5.9[B]	2^3-2^5
27B	112	0.4[B]	2^3-2^5
28C	157	3.4[B]	2^2-2^5
30C	143	3.7[B]	2^2-2^5
37C	186	8.4[C]	2^2-2^5
31C	113	7.9[C]	2^2-2^5
34C	172	6.2[C]	2^2-2^5
25C	132	6.8[A]	2^3-2^4
38C	135	4.9[C]	2^2-2^5

A: n = 2, B: n = 3, C: n = 4

The data are plotted along with the corresponding calibration standard data in Figures 23-26. The dose response for the sample diluates is essentially identical to the dose response for calibration standards.

In addition, it can be seen that the RSDs for each of the parallel analyses are not distinguishable from analytical variation. Hence it can be concluded that no significant matrix effects enter into the response of the PCB ELISA, for the samples obtained at the Allied Paper/Portage Creek/Kalamazoo River Superfund site.

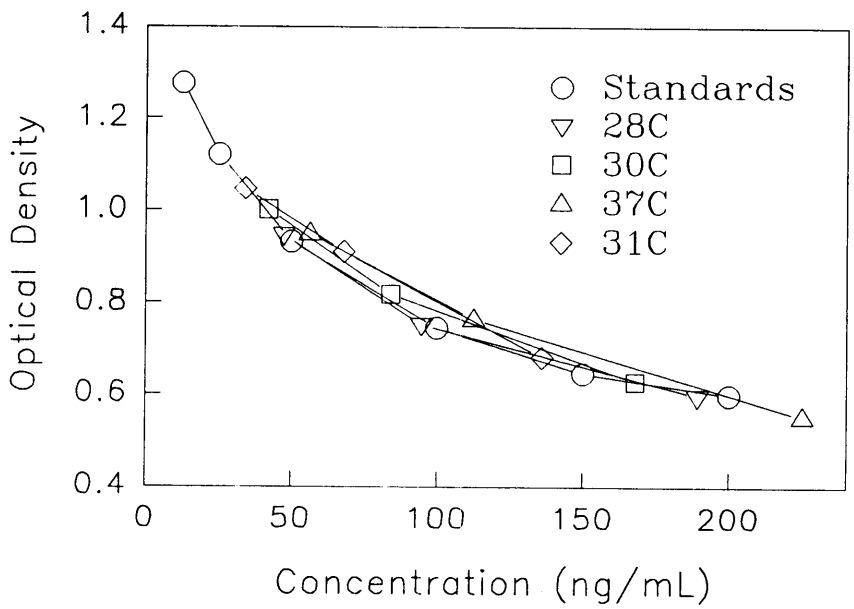

Figure 23. Dose response for serially diluted Allied Paper/Portage Creek samples, group 1.

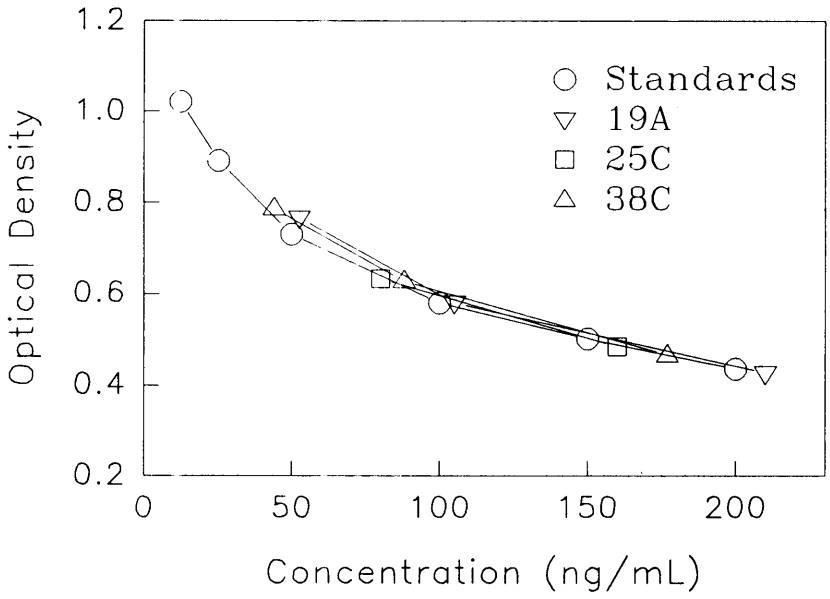

Figure 24. Dose response for serially diluted Allied Paper/Portage Creek samples, group 2.

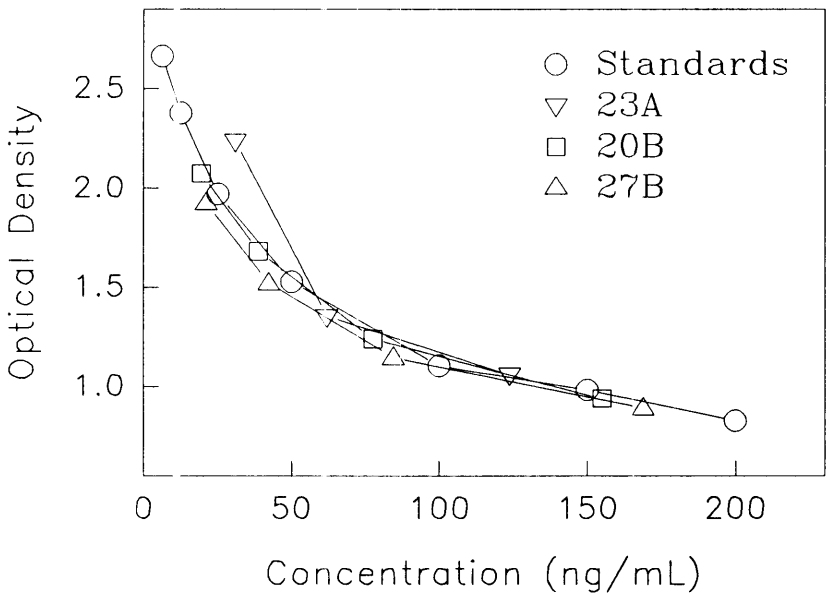

Figure 25. Dose response for serially diluted Allied Paper/Portage Creek samples, group 3.

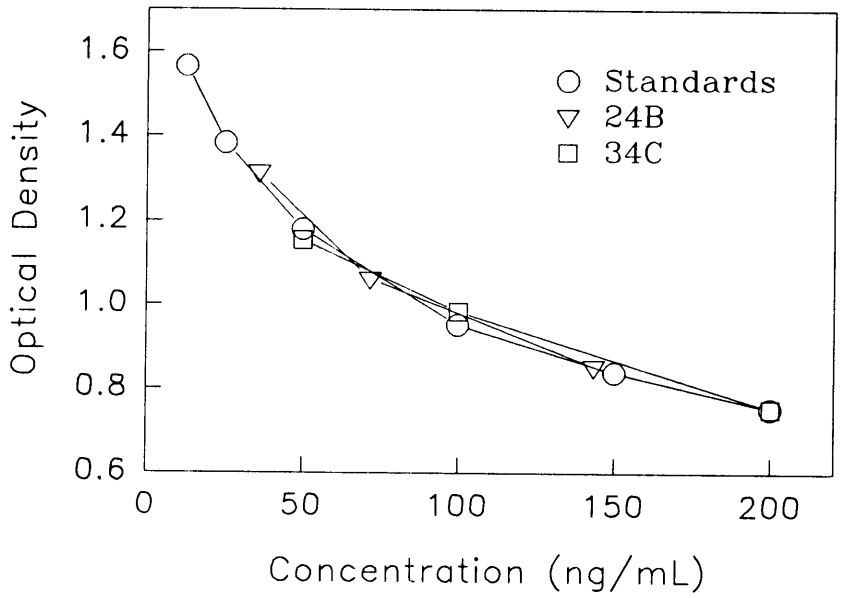

Figure 26. Dose response for serially diluted Allied Paper/Portage Creek samples, group 4.

Parallelism Results for NEIC Samples--

The NEIC samples were extracted and analyzed as described. During this analysis, only two data points fell on the calibration curve for each sample, and thus, the variation between duplicates is expressed as the relative percent difference (RPD). The data are summarized in Table 12. With the exception of sample 03-008-88-02-S, it can be seen that error for the diluates is essentially within the bounds of assay error, hence it can be concluded that no matrix effects were observed for the NEIC samples.

Spike Recovery for Real-World Samples

Approximately ten percent of the real-world samples were spiked, extracted and analyzed with the quantitative PCB plate ELISA, along with the corresponding non-spiked samples. An equivalent amount of the spiking solution itself was pipetted into volumes of methanol equal to the extraction volume. These latter solutions correspond to 100% extraction recovery, and were analyzed directly in the same ELISA as the sample spikes. In addition, the "100% recovery" solutions were taken through the entire extraction procedure to determine potential loss during the extraction manipulations.

Nine samples taken from the Kansas City SITE (to be discussed later) study were spiked with Aroclor 1248 using spiking procedure B. Five samples from the Allied Paper/Kalamazoo River/ Portage Creek Superfund site study were spiked with Aroclor 1242 using spiking procedure A.

TABLE 12. ELISA RESULTS FOR SERIALLY DILUTED NEIC EXTRACTS

Sample	ELISA Result (mg/Kg)	RPD
03-002-88-02-S	62.6	1.8
03-003-88-02-S	107	5.6
03-004-88-02-S	81	12.9
03-005-88-02-S	95.4	9.4
03-006-88-02-S	110	1.8
03-007-88-02-S	160	15.6
03-008-88-02-S	77.3	28.4
03-009-88-02-S	55.9	0.8

Results for Kansas City Soil Spikes--

The Kansas City samples were spiked with 250 µL of 1.1 x 10^{-4} g/mL standard solution of Aroclor 1248, which corresponds to a level of 5.5 mg/Kg in the soil. Three of the nine samples were aged approximately 1.5 months prior to extraction, the remainder overnight. The resulting extracts were serially diluted in methanol to bring them into the assay range. The measured concentrations for all serial dilutions within the assay range (typically 1:2 to 1:32) were averaged to arrive at a final concentration value. The expected value was calculated as the sum of expected spike value plus the measured value of the un-spiked sample. The results are summarized in Tables 13-15. Each Table represents a separate analytical standardization. The samples identified with asterisks were aged overnight.

TABLE 13. SPIKE RECOVERY OF AROCLOR 1248 FROM KANSAS CITY SOIL SAMPLES, SET ONE

Sample	Mean Aroclor Result (µg/Kg), RSD	Expected Value (µg/Kg)	Percent Spike Recovery
Spike Solution, Extracted	5722, 9.5%	5500	104.0
Spike Solution	6167, 10.8%	5500	112.0
KC13	937	NA	NA
KC22	365	NA	NA
KC13 Spike	6860, 8.4%	6437	106.5
KC22 Spike	5598, 18.0%	5864	95.5

TABLE 14. SPIKE RECOVERY OF AROCLOR 1248 FROM KANSAS CITY SOIL SAMPLES, SET TWO

Sample	Mean Aroclor Result (µg/Kg), RSD	Expected Value (µg/Kg)	Percent Spike Recovery
Spike Solution	5894, 9.7%	5500	107.2
KC31	324	NA	NA
KC43D	7587	NA	NA
KC81	931	NA	NA
KC31 Spike	6931, 5.5%	5824	119.0
KC43D Spike*	18704, 11.3%	13087	143.0
KC81 Spike*	5788, 10.8%	6431	90.0

TABLE 15. SPIKE RECOVERY OF AROCLOR 1248 FROM KANSAS CITY SOIL SAMPLES, SET THREE

Sample	Mean Aroclor Result (µg/Kg), RSD	Expected Value (µg/Kg)	Percent Spike Recovery
Spike Solution	4803, 12.6%	5500	87.3
KC83D	612	NA	NA
KC86D	2053	NA	NA
KC90D	1957	NA	NA
KC83D Spike*	6199, 5.1%	6112	101.4
KC86D Spike	7380, 8.5%	7553	97.7
KC90D Spike	6059, 11.8%	7457	81.3

Results for Allied Paper/Portage Creek/Kalamazoo River Soil Spikes--

The Kalamazoo samples were spiked with 500 µL of a 5 x 10^{-5} g/mL standard solution of Aroclor 1242, to give a PCB level of 5000 µg/Kg (for clean soils). The soils were aged two days wet and 4 days dry. The results after analysis by ELISA are summarized in Table 16.

TABLE 16. AROCLOR 1242 SPIKE RECOVERY FROM ALLIED PAPER/PORTAGE CREEK SAMPLES

Sample	Mean Aroclor Result (µg/Kg),	Expected Value (µg/Kg)	Percent Spike Recovery
Spike Solution	4732, 2.7%	5000	94.6
5A	464	NA	NA
12A	7659	NA	NA
4B	960	NA	NA
13B	9480	NA	NA
38C	3182	NA	NA
5A Spike	6685	5464	122.3
12A Spike	13166	12659	104.0
4B Spike	7309	5960	122.6
13B Spike	14997	14480	103.6
38C Spike	6630	8182	81.0

Summary of Spike Results for Real-World Samples--

For Aroclor 1248, spiked into real world clay samples at the 5.5 mg/Kg level, and subsequently analyzed by ELISA, it was found that the mean recovery was 104.3%, with a RSD of 17%. For Aroclor 1242, spiked into real-world soil, waste paper, and sediment samples at the 5 mg/Kg level, and subsequently analyzed by ELISA, it was found that the mean recovery was 106.7%, with a RSD of 14%.

Of course, recovery for extracts as determined by ELISA is confounded by the precision and accuracy of the ELISA itself. Based on recoveries determined for all spike solution analyses (n = 5), it was found that the mean recovery or accuracy of the ELISA at this Aroclor concentration was 101.0%, with a RSD of 8.9%. The accuracy levels attained in both Aroclor 1248 and Aroclor 1242 extractions are nearly identical to the accuracy of the spike solution recovery, thus it can be inferred that the RSD for the extract analyses is additive. The RSD for the extraction itself, then, is roughly $\leq$ 8%, a result which is within a factor of two of that obtained for extraction of the C-14 spikes.

The results obtained during the spike recovery studies demonstrate that the assay produced accurate determinations of spiked soil extracts. Further, the results demonstrate good extraction performance, at least for fortified real-world samples. Finally, accurate spike recovery is an additional demonstration of freedom from matrix effects for the real-world samples.

RESULTS FOR DUPLICATE ANALYSES

A statistically large subset of sample extracts was subjected to independent re-analysis in order to obtain a measure of reproducibility of the quantitative PCB plate ELISA . The population of data actually consists of three sub-populations: 1) Kansas City SITE samples re-assayed after greater than 3 months between analysis 2) Kansas City SITE samples re-assayed after several days and 3) Allied Paper/Portage Creek/Kalamazoo River samples re-assayed about 2 months later. The data are summarized in Table 17.

TABLE 17. ELISA RESULTS FOR DUPLICATE ANALYSES

Sample	Result 1 (mg/Kg)	Result 2 (mg/Kg)
KC71*	0.057	0.044
KC31	0.148	0.324
KC96*	0.155	0.055
KC57*	0.171	0.112
KC97*	0.173	0.141
KC87*	0.199	0.115
KC87D*	0.235	0.136
5A	0.601	0.464
KC81	0.707	0.930
KC83	0.818	0.777
4B	0.965	0.960
KC56	0.989	0.924
KC83D	1.02	0.612
KC39	1.11	0.968
KC22D	1.15	0.406
KC68	1.20	1.25
KC60D	1.37	1.59
KC53	1.63	1.71
KC13	1.64	0.936
KC22	1.90	0.364
KC42	2.14	1.51
KC90D	2.23	1.96
KC86D	2.36	2.053

Sample	Result 1 (mg/Kg)	Result 2 (mg/Kg)
KC17	2.5	2.80
KC98*	2.89	2.644
KC106*	3.56	4.97
KC55*	4.77	5.34
12A	6.82	7.66
KC43D	7.18	7.59
KC112*	112	88.0
KC103*	120	94.0

*Time between re-run < 4 days.

The duplicates are plotted against each other in Figure 27, excluding the two highest level samples, which statistically skew the regression line. The slope approaches unity, with a correlation coefficient of 0.95, indicating a high degree of reproducibility between duplicate analyses, most of which were separated in time by several months.

ELISA ANALYSIS OF REAL-WORLD SAMPLES

The final stage of the current project entailed the analysis of three sets of samples obtained from EPA SITE demonstrations, EMSL-LV Technical Support Center demonstrations and regulatory activities. During the design stage of the project, it was decided that three sets of samples of widely varying matrix composition should be employed in order to demonstrate wide applicability of the quantitative PCB plate ELISA based analytical procedure. The samples were collected as splits and extracted and analyzed according to the procedures outlined in the present report. The analytical results obtained by ELISA were compared to analytical data collected for the same samples using standard reference methods, as outlined below.

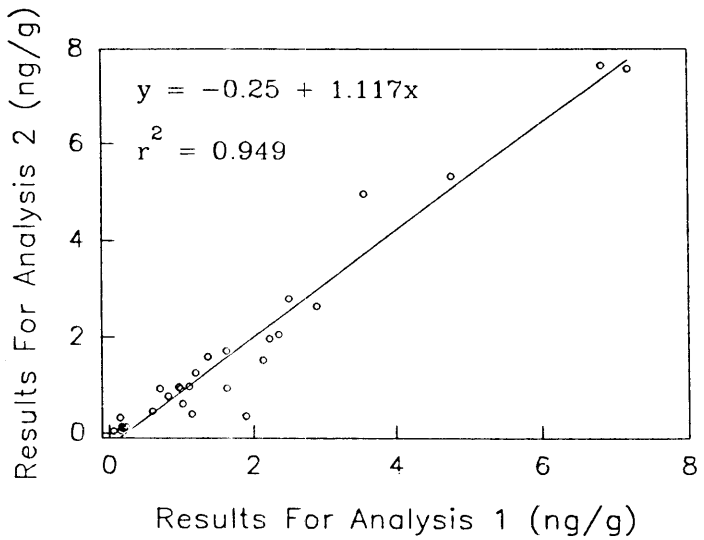

Figure 27. Duplicate ELISA analyses of Kansas City samples.

Analysis of Kansas City Site Samples

The first set of samples to be considered were collected in conjunction with an EPA Superfund Innovative Technology Evaluation (SITE) of field methods for PCBs. The location chosen was a U.S. Department of Energy (DOE) facility located about 20 miles south of downtown Kansas City, Missouri. The facility has been used since 1949 for the manufacture of various non-nuclear components destined for use in nuclear weaponry systems. The study site is located in a former channel of Indian creek, and contains a former storm water outfall that discharged from the DOE facility into the creek. During the early 1970's, Indian Creek was rerouted as part of a flood control project, and the former channel was covered with about 10 feet of fill.

In early 1988 sampling was conducted and PCBs were detected in the soil around the site. The contamination is believed to have occurred in the 1960's and 1970's as the result of occasional spills of a Therminol heat-transfer unit that subsequently drained into the outfall. In 1989, the DOE conducted a Resource Conservation and Recovery Act (RCRA) facility investigation (RFI) and corrective measures study (CMS), (U.S. Department of Energy, 1989), and as a result, the PCB concentrations are well mapped spatially at the site. The soil is contaminated with a wide range of PCB concentrations, from non-detect below 0.16 µg/Kg up to 9,680 mg/Kg.

Sediments overlying the bedrock consist of soft, dark brown to gray, homogeneous, medium to high plasticity, moist silty clay with traces of fine sand. This material varies in depth from 7 to 15 feet, and appears to have low permeability.

Description of Site Design Factors Relevant to the Plate ELISA--

The PCB SITE demonstration was designed as a comparative evaluation of four field screening technologies, all referenced to a common, accepted analytical method for PCB determination. Samples were collected at a site where PCB contamination, predominantly by Aroclors 1248 and 1242, had been well characterized in terms of concentration, allowing for collection of samples most suitable for challenging the efficacy of the technologies. Samples were collected and split five ways, one sample for each of the field technologies and one for the confirmatory method.

Sampling--

The basis for the experimental design was the control of with-in sample homogeneity. Samples on the order of 2 to 3 Kg were collected, physically homogenized and split among the four field screening technologies and the confirmatory method.

A sampling plan was designed such that coverage of a full range of concentrations was obtained, with an emphasis on the lower concentrations, near 10 mg/Kg. The plan called for the collection of the following samples: 20 samples from areas containing more than 1000 mg/Kg PCBs, 20 samples containing between 100 to 1000 mg/Kg, and 60 samples from areas containing less than 100 mg/Kg.

Prior to the start of the SITE demonstration, samples were collected from selected locations and sent to all involved technology developers as well as to EPA, EMSL-Las Vegas. Duplicate analyses were carried out using the field screening methods utilizing samples taken from the same sample container. Many of these duplicate analyses gave widely varying results, which, after checking for and finding no analytical error, led to the suspicion that homogeneity could be a potentially serious problem, particularly due to the fact that many of the samples were the consistency of modeling clay. Drying of the sample followed by thorough mixing of the resultant powder as typically carried out in classical laboratory methods was deemed to be unsuitable, as the method would no longer emulate

procedures carried out in field screening settings. Due to the fact that inter-method comparison was pivotal to the SITE study design, further measures were needed.

Due to the concern with the effects of possible sample inhomogeneity, it was decided that field duplicates would be randomly generated at a rate of about 20 to 25%. These field duplicates were taken as splits of the initially collected sample, and are therefore essentially (or should be) equivalent to the splits taken for the CLP analysis and ELISA analysis. The field duplicates were subjected to analysis by the quantitative PCB plate ELISA as well as by the CLP method. Analysis of such a relatively large number of duplicates by the reference method was specified in order to allow a statistically meaningful measure of the success in sample homogenization.

Reference Method--

The method used for obtaining reference data was the EPA Contract Laboratory Program (CLP) method outlined in the CLP Statement of Work (SOW) for Organics Analysis (U.S. Environmental Protection Agency, 1990a). This method is a gas chromatography method employing an electron capture detector. PCB extraction is carried out via Soxhlet extraction using a hexane/acetone mixture. The current study is intended to be a comparative study between the plate ELISA results and those obtained by the accepted standard method. As such, the CLP method results are taken to be the "true" values.

Results for Kansas City Site Samples--

Of the 142 samples collected during the SITE demonstration, 110 passed QA/QC requirements during CLP analysis. Samples from the set of 110 samples were selected at random and analyzed over a period of six weeks using the ELISA method as outlined previously. The method was calibrated with Aroclor 1248 and at least two soil SRM extracts were analyzed concurrently (typically, four soil extracts were run concurrently). The results for the ELISA analysis are tabulated in Table 18, along with the corresponding results by the CLP lab. CLP method detection limit was reported as 0.033 mg/Kg. Error for the ELISA represents ± 2 standard deviations, as derived from the standard deviation for 1248 SRM analysis.

The error for the CLP method (which is a derivative of SW-846 Method 8080/81) is harder to estimate. According to SW-846 Method 8080, the standard deviation, s, for determination of Aroclor 1248 in solution by a single analyst is ~0.17 X, where X is the average analytical result in µg/L (U.S. EPA, Office of Solid Waste and Emergency Response, 1986). The analytical error (± 2 standard deviations) for the CLP method results will thus be closely approximated as ± 34% of the reported value. It is important to note that these measures of variation are for the measurement stage of the method, not the entire method inclusive of the extractions.

TABLE 18. ELISA AND CLP RESULTS FOR KANSAS CITY SOIL SAMPLES

Sample ID	ELISA RESULT (mg/Kg)	ELISA ERROR (mg/Kg)	CLP RESULT (mg/Kg)	CLP ERROR (mg/Kg)
KC46D	0.224	0.067	<0.033	0.011
KC46	0.098	0.043	<0.033	0.011
KC49	0.165	0.022	<0.033	0.011
KC48	0.108	0.018	<0.033	0.011
KC45	0.031	0.002	<0.033	0.011

Sample ID	ELISA RESULT (mg/Kg)	ELISA ERROR (mg/Kg)	CLP RESULT (mg/Kg)	CLP ERROR (mg/Kg)
KC35	0.020	0.005	<0.033	0.011
KC99	0.048	0.008	<0.033	0.011
KC41	0.022	0.009	<0.033	0.011
KC35D	0.026	0.002	<0.033	0.011
KC92D	0.284	0.010	<0.033	0.011
KC87D	0.235	0.036	<0.033	0.011
KC71D	0.076	0.012	<0.033	0.011
KC82	0.029	0.024	<0.033	0.011
KC77	0.080	0.012	<0.033	0.011
KC70	0.055	0.014	<0.033	0.011
KC57	0.171	0.008	<0.033	0.011
KC51	0.253	0.062	<0.033	0.011
KC69D	0.134	0.084	<0.033	0.011
KC69	0.159	0.014	<0.033	0.011
KC109D	0.054	0.012	<0.033	0.011
KC109	0.263	0.070	<0.033	0.011
KC12	0.093	0.025	<0.033	0.011
KC110	0.025	0.054	<0.033	0.011
KC111	0.042	0.034	<0.033	0.011
KC24	0.071	0.006	0.055	0.019
KC27	0.052	0.028	0.057	0.019
KC21	0.156	0.022	0.063	0.021
KC87	0.199	0.044	0.076	0.026
KC67	0.120	0.028	0.081	0.028
KC03	0.288	0.042	0.114	0.039
KC14	0.234	0.018	0.180	0.061
KC105	0.034	0.006	0.210	0.071
KC28	0.113	0.037	0.216	0.073
KC82D	0.063	0.030	0.244	0.083

Sample ID	ELISA RESULT (mg/Kg)	ELISA ERROR (mg/Kg)	CLP RESULT (mg/Kg)	CLP ERROR (mg/Kg)
KC31	0.148	0.024	0.263	0.089
KC93	0.124	0.004	0.295	0.100
KC83D	1.020	0.104	0.413	0.140
KC81D	0.669	0.044	0.450	0.153
KC83	0.818	0.048	0.484	0.165
KC56	0.989	0.112	0.505	0.172
KC42	2.14	0.416	0.517	0.176
KC22	1.90	0.122	0.535	0.183
KC07	3.31	0.540	0.552	0.189
KC60D	1.37	0.366	0.577	0.196
KC01	4.41	0.160	0.593	0.202
KC39	1.11	0.128	0.676	0.230
KC06	2.89	0.266	0.679	0.231
KC58	2.50	0.224	0.681	0.232
KC81	0.707	0.014	0.687	0.234
KC22D	1.15	0.042	0.718	0.244
KC98D	2.09	0.104	0.825	0.281
KC53	1.63	0.112	0.958	0.326
KC90	2.28	0.162	1.01	0.343
KC84D	5.89	0.294	1.08	0.367
KC13	1.64	0.136	1.13	0.384
KC30	0.479	0.059	1.15	0.391
KC84	5.90	0.119	1.16	0.394
KC98	2.89	0.342	1.17	0.398
KC92	1.69	0.290	1.21	0.411
KC86D	2.36	0.184	1.25	0.425
KC05	10.1	0.502	1.37	0.467
KC90D	2.23	0.098	1.40	0.476
KC86	2.68	0.142	1.42	0.483

Sample ID	ELISA RESULT (mg/Kg)	ELISA ERROR (mg/Kg)	CLP RESULT (mg/Kg)	CLP ERROR (mg/Kg)
KC02	7.90	0.130	1.50	0.510
KC43D	7.18	0.198	1.74	0.592
KC88D	4.26	0.310	1.77	0.602
KC102D	6.82	0.334	1.77	0.602
KC26	1.68	0.342	1.96	0.666
KC66	4.45	0.466	1.98	0.673
KC08	24.8	1.578	2.00	0.680
KC78	5.90	0.078	2.27	0.772
KC62	2.54	0.674	2.35	0.799
KC55	4.77	0.152	2.40	0.816
KC106	3.56	1.77	2.50	0.850
KC17	2.50	0.213	2.55	0.867
KC88	3.02	0.170	2.70	0.918
KC65	33.7	15.2	3.08	1.05
KC50	13.6	2.37	3.60	1.22
KC80	7.85	0.148	3.77	1.28
KC52	2.72	1.06	4.21	1.43
KC40	13.9	4.84	4.25	1.44
KC50D	12.6	1.19	4.41	1.50
KC19	8.49	0.577	6.70	2.28
KC104	28.1	5.26	7.66	2.60
KC59	11.2	1.95	7.86	2.67
KC15	6.23	0.836	9.13	3.10
KC25	54.8	5.66	11.7	3.98
KC74	14.5	2.18	13.3	4.52
KC73	33.8	11.3	15.8	5.37
KC95	47.2	11.2	17.5	5.95
KC23	18.7	3.24	20.8	7.07
KC75	14.6	1.60	23.0	7.82

Sample ID	ELISA RESULT (mg/Kg)	ELISA ERROR (mg/Kg)	CLP RESULT (mg/Kg)	CLP ERROR (mg/Kg)
KC34	20.0	5.70	34.0	11.6
KC79	68.9	5.60	42.8	14.6
KC89	25.7	1.45	45.0	15.3
KC18	127	30.2	45.2	15.4
KC76	84.8	3.75	46.7	15.9
KC32	15.7	3.50	47.6	16.2
KC100D	638	127	167	56.8
KC100	616	51.7	177	60.2
KC102	6.93	0.984	293	99.6
KC112	119	67.6	315	107.1
KC103	120	9.32	403	137
KC85	39.0	3.12	428	146
KC85D	415	131	465	158
KC61	570	12.3	580	197
KC36	2310	109	816	277
KC91	935	152	1630	554
KC91D	1160	264	1704	579
KC16	607	74.4	2110	717

Another, possibly more informative, representation of the results is given in Figures 28-31. Each grouping of two bars represents the ELISA result and the CLP result for a given sample. The data are grouped by concentration range to allow full axis expansion. The non-detects (< 0.033 mg/Kg) are depicted as 0.033 mg/Kg for convenience only.

The most striking trend in the results is that, generally speaking, the PCB levels, as measured by quantitative PCB plate ELISA, are higher than the corresponding value obtained by CLP analysis. Based on earlier discussions, it can be concluded that the ELISA itself is accurate. For Aroclor spiked directly into extraction solvent, it was seen that determinative accuracy was very high. The spike recovery values also show good accuracy and precision. Further, performance of the method as applied to the two Aroclor 1248 soil SRMs shows that accuracy of the total method is high.

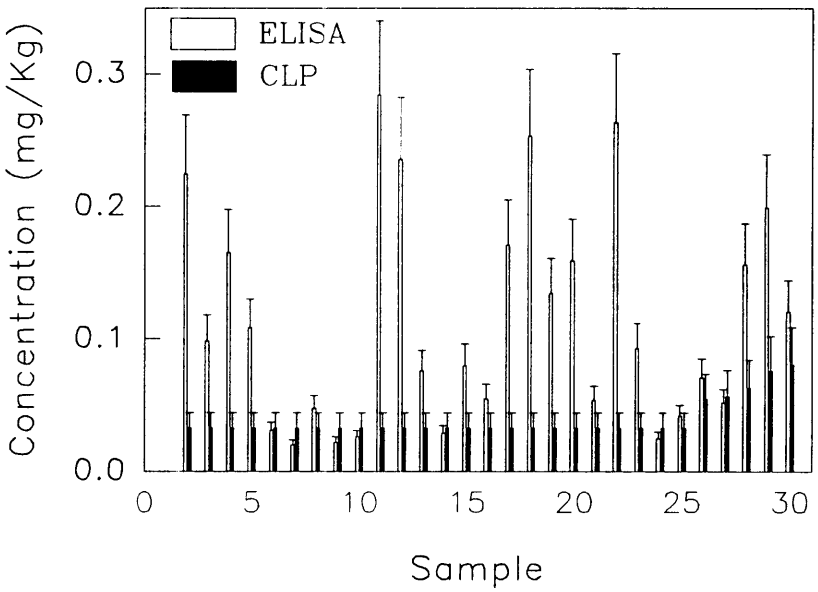

Figure 28. ELISA and CLP results for Kansas City samples, group 1.

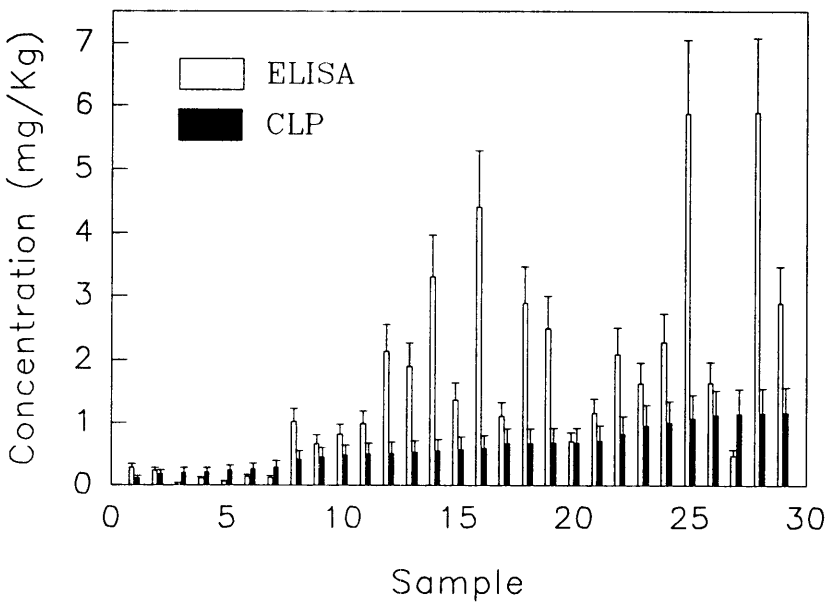

Figure 29. ELISA and CLP results for Kansas City samples, group 2.

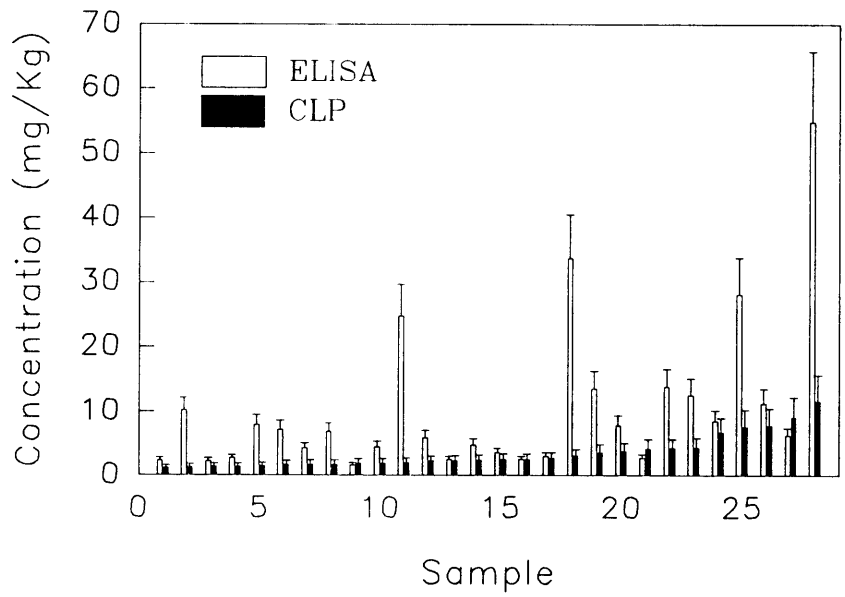

Figure 30. ELISA and CLP results for Kansas City samples, group 3.

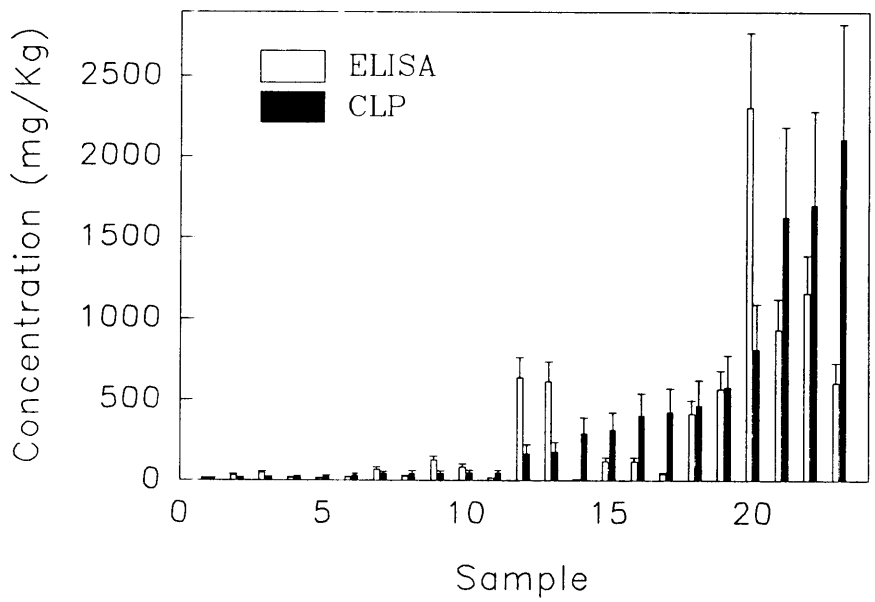

Figure 31. ELISA and CLP results for Kansas City samples, group 4.

The data sets can be compared using a paired t-test. Careful consideration must be given to the proper application of this statistic as applied to a data set such as the results for the Kansas City samples, where the numerical values of the data points extend over almost 5 orders of magnitude. It is necessary to use a modified form of the t-test:

$$t = \frac{\bar{d} - \delta}{\sqrt{s^2/n}}$$

Where

$\bar{d}$ = is the mean of relative sample differences = Σ (($Result_{ELISA}$-$Result_{CLP}$)/$Result_{CLP}$)
δ = is the population variance mean difference (0 if the data sets are equivalent)
s^2 = is the estimated variance of the differences
$Result_{ELISA}$ = ELISA result for sample i)
$Result_{CLP}$ = CLP result for sample i)
n = number of samples

Assuming the CLP result to be the "true" value, division of the difference between the sample pairs by the CLP result converts the difference into a relative difference. If the two data sets are equivalent, the relative mean difference will be zero, analogous to the case where the mean difference should be zero as well.

If the mean differences are not converted to relative differences, the magnitude of the mean differences will be larger than the magnitude of most of the results themselves, due to the sample distribution. The differences for the lower concentration samples will become statistically insignificant relative to the differences for the high concentration samples, and hence, the t statistic will be skewed by the few high level samples. Carrying out the modified t-test calculation, we get:

$\bar{d}$ = 1.5469
s^2 = 2.2502
n = 110
t = 1.08

for 109 degrees of freedom, $p << 0.005$, thus, statistically, the two data sets are not equivalent. The mean relative difference shows that the ELISA results are on the average greater than the CLP results by a factor of about 2.5.

In addition to the ELISA analysis of methanol shake extracts described above, a subset consisting of 23 samples was randomly selected, extracted using supercritical fluid extraction, and then analyzed by ELISA. An intriguing picture begins to emerge when one considers the ELISA results for methanol shake extracts as compared to both the ELISA results for supercritical fluid extracts and CLP results. The data are summarized in Table 19, and the SFE-ELISA data are plotted against the corresponding CLP data in Figure 32.

TABLE 19. ELISA, SFE-ELISA AND CLP RESULTS FOR KANSAS CITY SOIL SAMPLES

Sample	MeOH Shake ELISA (mg/Kg)	SFE-ELISA (mg/Kg)	SFE-ELISA ERROR (mg/Kg)	CLP (mg/Kg)
KC12	0.093	0.039	0.002	<0.033
KC99	0.048	0.013	0.001	<0.033
KC41	0.022	0.035	0.003	<0.033
KC27	0.052	0.028	0.002	0.057
KC03	0.276	0.536	0.015	0.114
KC14	0.234	0.166	0.011	0.180
KC31	0.148	0.387	0.024	0.263
KC83	0.818	0.363	0.009	0.484
KC56	0.989	0.304	0.013	0.505
KC39	1.11	0.436	0.014	0.676
KC13	1.14	1.25	0.029	1.13
KC30	0.479	0.105	0.006	1.15
KC05	16.9	12.0	0.675	1.37
KC90D	2.23	1.52	0.274	1.40
KC86	2.68	1.61	0.198	1.42
KC26	1.68	0.638	0.074	1.96
KC17	2.50	1.21	0.072	2.55
KC88	3.02	1.58	0.102	2.70
KC40	13.9	8.40	0.277	4.25
KC33	11.2	5.40	0.149	6.00
KC19	8.49	4.49	0.254	6.70
KC25	59.9	11.6	1.36	11.7
KC23	18.7	9.45	0.794	20.8

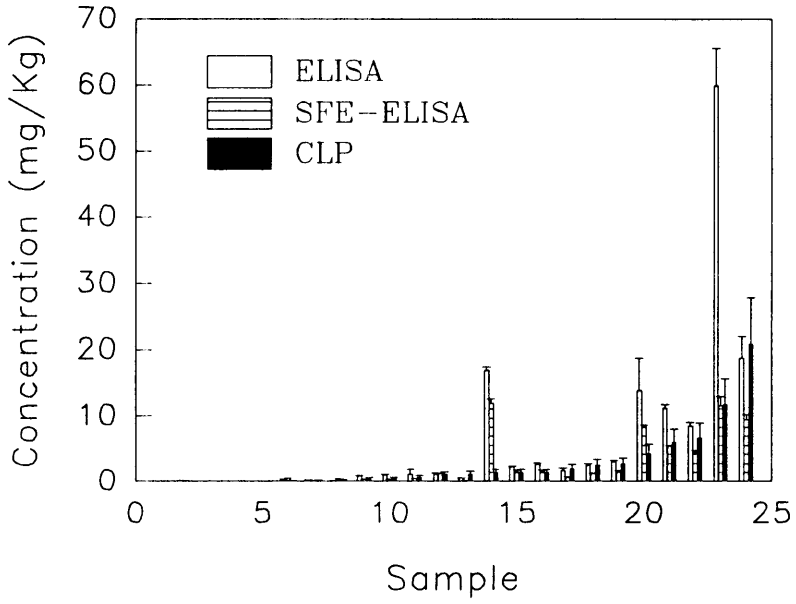

Figure 32. SFE-ELISA and CLP results for re-extracted Kansas City Samples.

In general, the SFE-ELISA gave results which are lower than the results for ELISA analysis of samples extracted with methanol. The SFE-ELISA results, in general, are convergent with the CLP results.

A paired t-test was carried out on the SFE-ELISA data and the CLP data with the null hypothesis that the data sets are equivalent. Carrying out the calculation with this hypothesis gives:

$t = 0.8729$
$p = 0.39$

The two data sets are thus equivalent by the t-test. In contrast, if the methanol shake extraction/ELISA data and the CLP data for the same samples are compared using the paired t-test, a t-value of 2.118 is obtained, with a corresponding probability of only 0.046. The methanol shake/ELISA results and the CLP results are not equivalent to the 0.05 level. The SFE-ELISA data results are equivalent to the CLP results, with a mean RPD between SFE-ELISA results and CLP results of -14%. The mean RPD between methanol shake/ELISA and CLP results, in comparison, is 35%.

Given the fact that the ELISA performance itself is well characterized statistically (RSD $\leq$ 10% for replicate inter-assay analyses, based on Aroclor 1248 SRM data), it is clear that the difference between the two ELISA results is due to the extraction procedure. Of course, sample homogeneity will play a role, but it could be expected to be random with regard to PCB level. Clearly, this is not the case.

As discussed earlier, extraction efficiency using methanol is expected to be greater than that for hexane, or hexane/acetone mixtures (Spittler, 1986). The CLP method specifies acetone/hexane, a solvent system which apparently was optimized for the suite of analytes covered by the method (non-volatile organochlorines and pesticides).

Based on this knowledge, it can be hypothesized that the results for the ELISA analyses and the CLP analyses differ, in large part, due to the different extraction procedures employed. For example, the lower extraction efficiency of the hexane/acetone mixture could come into play for that subset of the Kansas City samples which are more "difficult" to extract than the others. The ideal case for comparison of ELISA data to GC data would entail co-analysis of extract splits generated by whatever particular extraction method was employed. Unfortunately, this was beyond the scope of the current study.

Analysis of Allied Paper/Portage Creek/Kalamazoo River Superfund Site Samples

Background--

The samples analyzed with the quantitative PCB plate ELISA in the current study were collected as part of an EPA Technical Support demonstration project referred to as the "Field Screening Technologies Analysis of Polychlorinated Biphenyls At The Allied Paper/Portage Creek/Kalamazoo River Superfund Site, Kalamazoo, Michigan." The quantitative PCB plate ELISA was not an integral component of the demonstration project, rather, the participants in the project generously agreed to collect sample splits which were intended for use by investigators working in the Immunochemistry Program at EMSL-LV.

The Allied Paper/Portage Creek/Kalamazoo River Superfund Site is located in the southeast corner of Michigan's lower peninsula. The site covers 35 miles of the Kalamazoo river, from the confluence with Portage Creek, and approximately 3 miles of Portage creek. PCBs are the main contaminant of concern at this site, and according to the Michigan Department of Natural Resources (MDNR), are due mainly to carbonless copy paper recycling during the 1950's through the 1970's.

This site has been extensively characterized over the past several decades, and the soil types have been characterized as ranging from poorly to well drained soils that have loamy to sandy and loamy subsoils formed in glacial outwash (Blasland and Bouck, 1991). During pre-demonstration activities, it was determined that Aroclor 1242 was the main Aroclor of interest in the context of the field demonstration. Three types of sample matrices were collected: soil, sediment and paper waste. For further detail, refer to the Demonstration and Quality Assurance Project Plan for the "Field Screening Technologies Analysis of Polychlorinated Biphenyls At The Allied Paper/Portage Creek/Kalamazoo River Superfund Site, Kalamazoo, Michigan," available through the Technology Support Center, Environmental Monitoring Systems Laboratory, Las Vegas, NV (U.S. EPA, 1992a).

Analysis of Samples by Quantitative PCB Plate ELISA--

Thirty-eight samples were extracted and analyzed by quantitative PCB plate ELISA according to procedures described. Each analytical run included analysis of the five commercially obtained soil SRMs as described in Section 4. Separate splits of all the samples were analyzed by the MDNR using SW-846 Method 8081 according to quality control/quality assurance measures specified by that method (U.S. EPA, Office of Solid Waste and Emergency Response, 1992c). The samples are designated with a letter prefix A, B, or C and a number. The A corresponds to river sediment, B to soil, and C to paper waste and the numbers, to a specific sampling location. These locations are arbitrary in terms of the present study.

The results are summarized in Table 20:

TABLE 20. ELISA AND SW-846 METHOD 8081 RESULTS FOR ALLIED PAPER/PORTAGE CREEK/KALAMAZOO RIVER SOIL SAMPLES

Sample ID	ELISA Result (mg/Kg)	ELISA Error (mg/Kg)	Method 8081 Result (mg/Kg)	Method 8081 Error (mg/Kg)
1B	1.00	0.201	<0.5	0.170
2B	0.204	0.041	<0.5	0.170
3B	1.58	0.316	0.540	0.184
4B	1.81	0.362	0.600	0.204
5A	0.552	0.110	0.630	0.214
6B	2.73	0.546	1.02	0.347
7B	2.41	0.482	2.60	0.884
8A	2.90	0.580	2.75	0.935
9A	7.93	1.59	3.60	1.22
10B	3.23	0.645	6.00	2.04
11B	11.0	2.20	8.90	3.03
12A	6.82	1.36	11.0	3.74
13B	9.48	1.90	11.6	3.94
14A	8.85	1.77	12.0	4.08
15A	12.5	2.51	17.9	6.09
16A	17.4	3.49	21	7.14
17A	10.8	2.16	34.0	11.6
18B	67.2	13.4	74.0	25.2
19A	26.0	5.20	81.0	27.5
20B	48.0	9.61	88.0	29.9
21B	37.8	7.55	95.0	32.3
22C	28.5	5.69	106	36.0
23A	23.3	4.65	107	36.4
24B	146	29.2	136	46.2
25C	47.7	9.53	139	47.3
26C	91.4	18.3	140	47.6
27B	56.3	11.3	160	54.4

Sample ID	ELISA Result (mg/Kg)	ELISA Error (mg/Kg)	Method 8081 Result (mg/Kg)	Method 8081 Error (mg/Kg)
28C	44.6	8.91	170	57.8
29C	240	48.0	180	61.2
30C	39.9	7.98	180	61.2
31C	41.2	8.23	186	63.2
32C	120	24.0	190	64.6
33C	46.0	9.19	204	69.4
34C	122	24.4	240	81.6
35C	37.1	7.42	242	82.3
36C	60.6	12.1	260	88.4
37C	100	20.0	267	90.8
38C	78.7	15.7	268	91.1

The MDNR laboratory detection limit was reported as 0.5 mg/Kg. Again, method error for ELISA is taken as 2 standard deviations derived from soil SRM analysis. Method 8081 error is 34%, as discussed earlier. The data are summarized graphically in Figures 33 and 34. The non-detects (< 0.5 mg/Kg) are depicted as 0.5 mg/Kg for convenience only.

Figure 33 shows that for the "lower level" samples (< 30 mg/Kg), there is close agreement between the PCB plate ELISA results and the Method 8081 results; most of the ELISA and Method 8081 results overlap within the error limits of the respective methods. Using the modified paired t-test with the null hypothesis that the data sets are equivalent gives:

$t = 1.53$
$p = 0.14$

The two sets of "low level" results are thus equivalent by the t-test at the 0.14 level.

In contrast, the results for the "higher level" samples (>30 mg/Kg) show a different trend. Generally, the PCB ELISA results are significantly lower than the Method 8081 results, by up to a factor of 6.5, as can be seen in Figure 34. Performing the paired t-test on these data gives:

$t = -8.46$
$p << 0.001$

It was reasoned that since the problem lies mainly with the high level samples, there may be a non-equilibrium partitioning with the shake extraction procedure. Contrasted with Soxhlet extraction, which essentially provides multi-stage extraction, the shake procedure provides only a one-stage equilibrium partitioning between the solid sample and solution. In the case of the high level samples, the quantity of non-extracted PCBs heldup on the solid sample matrix could be significant.

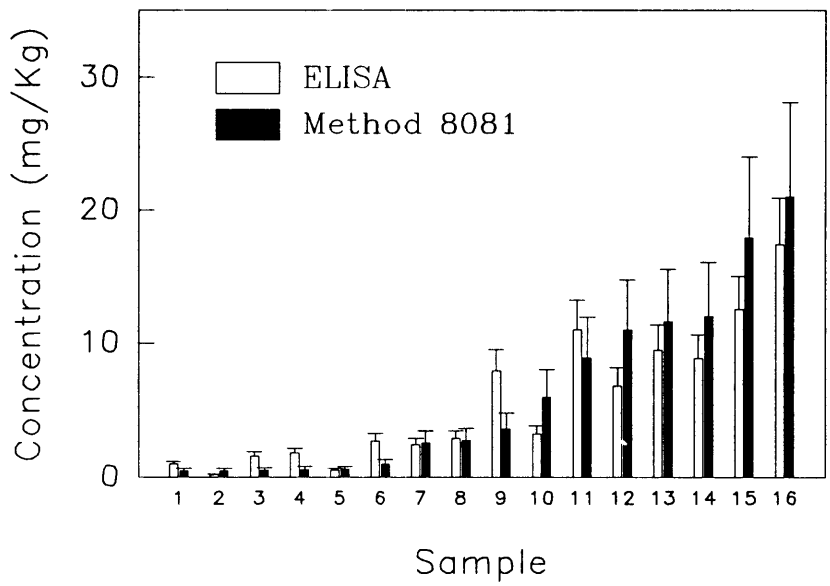

Figure 33. ELISA and SW-846 Method 8081 results for Allied Paper/Portage Creek/Kalamazoo River samples, group 1.

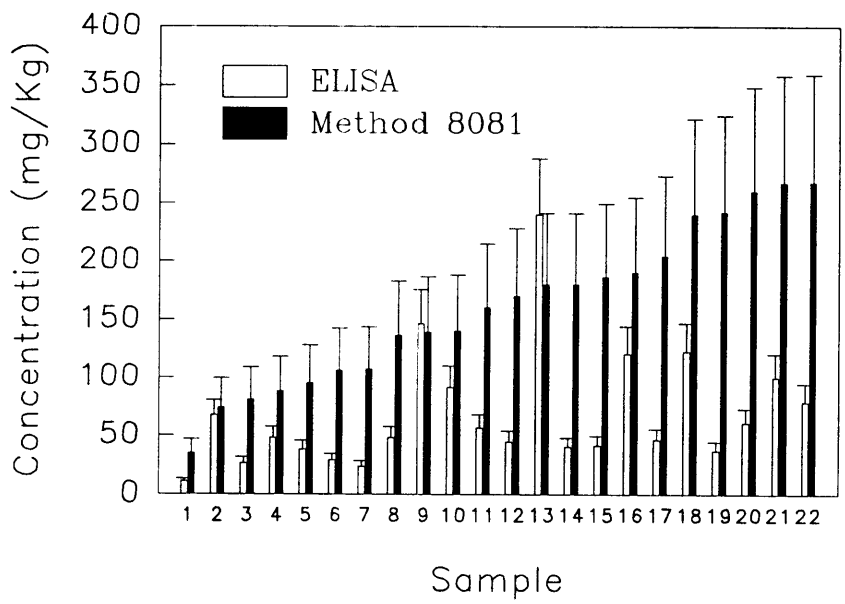

Figure 34. ELISA and SW-846 Method 8081 results for Allied Paper/Portage Creek/Kalamazoo River samples, group 2.

In an effort to verify this hypothesis, the high level samples were re-extracted using the Soxhlet procedure specified in SW-846 Method 3540A (U.S. EPA, 1990b) with the exception that methanol, rather than hexane/acetone, was used. After overnight extraction, the methanolic extracts were serially diluted and analyzed by the ELISA. The data are summarized in Table 21.

TABLE 21. ELISA RESULTS FOR SOXHLET EXTRACTS AND METHOD 8081 RESULTS FOR ALLIED PAPER/PORTAGE CREEK/KALAMAZOO RIVER SOIL SAMPLES

Sample	ELISA Result (mg/Kg)	ELISA Error (mg/Kg)	Method 8081 Result (mg/Kg)	Method 8081 Error (mg/Kg)
19A	94.5	18.9	81.0	27.5
20B	66.5	13.3	88.0	29.9
22C	104	20.9	106	36.0
23A	66.5	13.3	107	36.4
25C	132	26.4	139	47.3
27B	112	22.5	160	54.4
28C	157	31.5	170	57.8
30C	143	28.6	180	61.2
31C	113	22.6	186	63.2
33C	131	26.3	204	69.4
36C	196	39.2	260	88.4
38C	173	34.6	268	91.1
37C	186	37.2	267	90.8

The data are represented graphically in Figure 35.

Each group of three bars represent ELISA results for the methanol shake extraction, ELISA results for the methanolic Soxhlet extracts and the Method 8081 results. Two points are evident. First, all ELISA results for the Soxhlet extracts fall within 2 standard deviations of error for the Method 8081 results. Analytically, the Soxhlet/ELISA and the Method 8081 results appear to be equivalent. Second, the differences in ELISA results (shake versus Soxhlet) are a consequence of extraction procedure only, as the two sets of results overlap within the error limits of the respective methods. The only difference in the extraction was vigor, rather than solvent or sample clean-up etc., and thus the difference in ELISA results is due almost solely to extraction method. These experiments demonstrate that, coupled to an appropriate sample preparation procedure, the ELISA is capable of providing highly accurate analytical data.

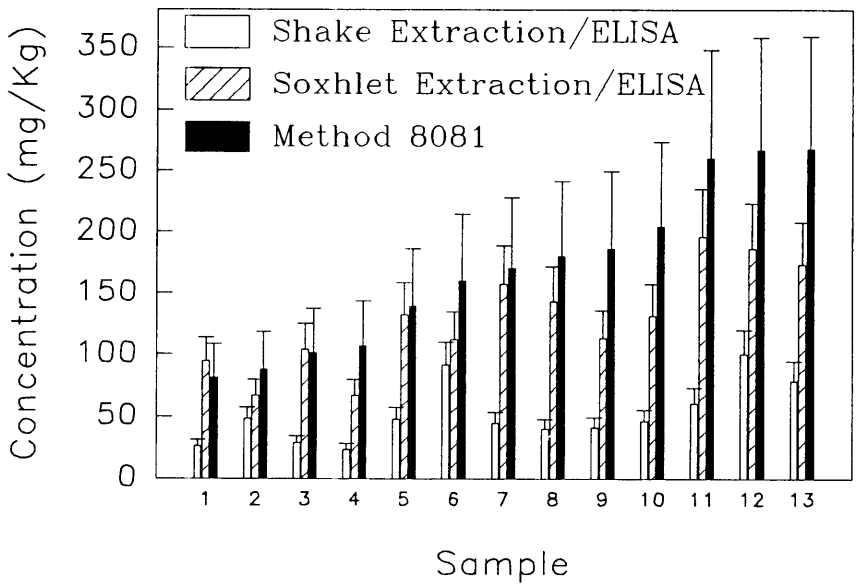

Figure 35. ELISA and SW-846 Method 8081 results for Soxhlet extracts of high level samples.

This conclusion that the Soxhlet/ELISA and Method 8081 data sets are equivalent was tested by carrying out the modified paired t-test on the data. Carrying out the calculation gives:

$\bar{d} = -0.21$
$s^2 = 0.029$
$t = -4.59$
$p < 0.005$

The hypothesis that the data sets are statistically equivalent must be rejected on the basis of the t-test. The failure of the test in this case is due in large part to the small variation seen in the relative difference values. The ELISA results are biased low, on the average, by a factor of about 0.83.

From a practical standpoint, a 17% bias is of little importance; inter-lab bias of such a magnitude is commonly observed even when using the same method. In the present case of comparing quantitative PCB plate ELISA data to Method 8081 data, not only is an inter-method comparison being made, but a confounding inter-lab comparison is being made as well. It can thus be concluded that for real-world use, the quantitative PCB plate ELISA provided accurate results when coupled with a rigorous extraction procedure.

The results for the Allied Paper/Portage Creek/Kalamazoo River Superfund site samples demonstrate the potential dangers of abbreviated extraction procedures. The 20 minute mechanical rocker shaking employed in the current work is more vigorous than many of the rapid hand shake extraction procedures common to the commercial ELISA based screening methods, yet the 20 minute mechanical shake extraction procedure was shown to have an extraction efficiency far lower than an overnight Soxhlet extraction using the same solvent. Perhaps many of the "false negative" results observed in field screening applications are due to poor extraction performance, rather than poor ELISA performance. It appears that the tough question of extraction validation may need to be more adequately addressed.

Analysis of EPA NEIC Samples

Background--

Samples employed during this phase of the current study were obtained from the EPA National Enforcement and Investigation Center (NEIC) in Denver, Colorado. The sample splits were taken from archival samples which had already been subjected to analysis by NEIC chemists using the method described in "PCB Analytical Program Standard Operating Procedure" (Hill et al., 1989). This GC/ECD method is a modification of a previously published method (Bellar et al., 1982). Based on reference data provided by NEIC, single analyst standard deviation was calculated to be 18.5%, thus method error was taken to be ± 37%. Nine samples characterized as being contaminated with a mixture of Aroclors 1242/1254/1260 were obtained. The sample matrices ranged from soil to oily sludge.

Analytical Procedure--

The samples were extracted according to the described procedures. Extracts were serially diluted in 1 mL of methanol, typically up to 1:256 dilution. Aroclor 1242 was chosen as the calibrator. Samples were analyzed in triplicate wells according to the described procedures.

Results--

The results for ELISA and NEIC GC analysis are summarized in Table 22.

TABLE 22. ELISA AND NEIC RESULTS FOR NEIC SOIL SAMPLES

Sample	ELISA Result (mg/Kg)	ELISA Error (mg/Kg)	NEIC Results (mg/Kg)	NEIC Error (mg/Kg)
01-012-92-03-S	172	7.83	46.0	17.0
03-002-88-02-S	62.6	3.63	37.0	13.7
03-003-88-02-S	107	3.52	61.0	22.6
03-003-93-02-S	770	17.3	307-841	-
03-004-88-02-S	81	5.10	46.0	17.0
03-005-88-02-S	95.4	19.1	62.0	22.9
03-006-88-02-S	110	3.45	63.0	23.3
03-007-88-02-S	160	9.00	235	87.0
03-008-88-02-S	77.3	9.10	101	37.4
03-009-88-02-S	55.9	3.61	43.0	15.9

The data are represented graphically in Figure 36. The data point for sample 03-003-93-02 has been removed because of the large uncertainty of the NEIC result. On average, it can be seen that the ELISA values tend to run higher than the corresponding NEIC values. This is most likely not a matrix effect, as variation for sample diluates is low, and furthermore, the samples are not necessarily spatially related, and indeed, obvious diffences were observed between samples. Several samples were oily clays, whereas several of the other samples were moist sediments or soils.

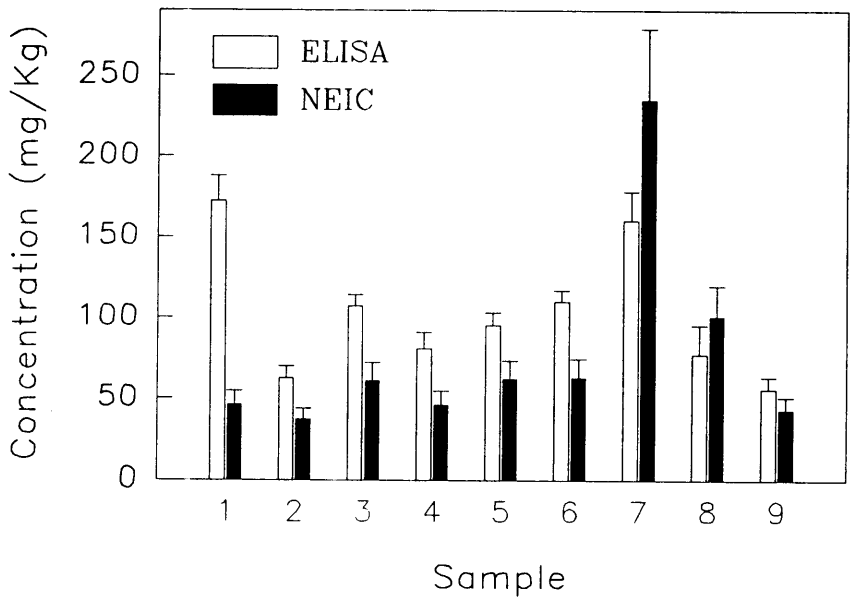

Figure 36. ELISA and NEIC results for NEIC samples.

A number of factors could come into play, the most important probably being the fact that, as reported by NEIC, a portion of the Aroclor mixture is Aroclor 1254 and 1260. As reported in Table 1, the ELISA is approximately 2 times as sensitive to Aroclor 1254 and about 1.6 times as sensitive to Aroclor 1260 relative to Aroclor 1242, thus the ELISA response for the NEIC samples is somewhere between a factor of 1 to 2, dependant upon the fractions of 1254 and 1260 present.

A paired t-test with the null hypothesis again being that two data sets are statistically equivalent gives:

$\bar{d} = 0.6640$
$\sigma^2 = 0.6948$
$t = 2.3894$
$p < 0.05$

The null hypothesis must thus be rejected; the ELISA results are, on the average, biased high by a factor of about 1.7, which is consistent with, but does not confirm the statements made in the proceeding paragraph. Calibration of the quantitative PCB plate ELISA with Aroclor 1254 would shift the curve such that measured Aroclor levels would be one half that reported using Aroclor 1242 calibration. The ELISA results would thus be biased only slightly low from the NEIC GC results.

These results are illustrative of a common problem in PCB calibration, whether for GC or ELISA analysis, namely, that of determining the most suitable Aroclor calibrant. The Aroclor chosen for calibration must emulate the actual composition of the samples. This is true whether the determinative method is by ELISA or GC.

Summary of Results for Real-World Samples

Two large sets of unrelated environmental samples obtained from Superfund sites were analyzed using a simple, shake extraction with methanol, followed by determination of PCBs with the quantitative PCB plate ELISA. In general, it was seen that the quantitative PCB plate ELISA results were statistically biased away from the corresponding results obtained using standard GC methods. In the case of one set of samples, the clays from Kansas City, the results were biased high, on the average, by a factor of approximately 2.5. For the second set of samples, the soil, sediment and paper waste samples from the Allied Paper/Portage Creek/Kalamazoo River site, the ELISA results were biased low, on the average, by a factor of 0.83.

Following re-extraction of a randomly selected sub-set of the Kansas City samples with supercritical fluid extraction, followed by measurement of PCBs with the quantitative PCB plate ELISA, it was found that the ELISA results for this sub-set of samples were essentially equivalent to the GC results. This result indicates that the quantitative PCB plate ELISA effectively measured PCB levels in the Kansas City clay samples when applied to appropriately prepared sample extracts.

The quantitative PCB plate ELISA performed well for shake extracts of Allied Paper/Portage Creek/Kalamazoo River samples with PCB levels below 30 mg/Kg; the ELISA results were not statistically different from the GC results for this sub-set of the samples. In contrast, the PCB plate ELISA measured significantly lower PCB levels than expected, based on the GC results, in methanol shake extracts of samples with PCB levels greater than 30 mg/Kg.

This latter sub-set of samples were re-extracted using a more rigorous, overnight extraction procedure, and re-analyzed using the quantitative PCB plate ELISA. The ELISA results overlap the GC results, within the error limits of the respective methods. The ELISA results were still biased low, but on the average by only about 20%, which is of little practical concern considering that this comparison of results is actually an inter-lab, inter-method comparison. The ELISA results for the Allied Paper/Portage Creek/Kalamazoo River samples demonstrated that the quantitative PCB plate ELISA can provide accurate determination of PCBs in soil, sediment and paper waste provided it is used in conjunction with adequate sample preparation procedures.

Assorted environmental samples obtained form NEIC were analyzed using the quantitative PCB plate ELISA calibrated with Aroclor 1242 standards. The results were biased high, on the average, by a factor of 1.7 relative to the GC results. It was later reported by NEIC that the samples contained mixtures of Aroclors 1242/1254/1260. The calibration standards were thus somewhat inappropriate for these particular samples.

Based on performance data for the quantitative PCB ELISA, it can be stated that simply by switching to Aroclor 1254 calibration standards, the quantitative PCB plate ELISA would have provided results which, on the average, would be biased low by 30%, which is acceptable performance for inter-method comparisons based on inter-lab data. This is particularity true for standard methods intended for analysis of PCBs.

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