

A field validation study of a PAH biosensor

LA Clarke & Son, Spotsylvania, VA

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Summary

Water samples of varying PAH concentrations were collected from 8 wells at the LA Clarke & Son site in Spotsylvania, VA. The samples were analyzed with an antibody-based biosensor and concentrations validated by GC-MS. The biosensor was capable of analyzing samples on-site and within 30 minutes of sample acquisition. The results show that the biosensor adequately discerned PAH concentrations in well water samples.

Background

Presently, immunoassays are being developed and employed as alternatives to conventional analytical chemistry for environmental sampling. The major advantages of immunoassays are that they require little or no sample manipulation, can be performed quickly (minutes to hours) and can be performed on-site. As such, we are in the process of developing and testing a portable antibody-based biosensor for the rapid assessment of 3-5 ring polycyclic aromatic hydrocarbons (PAHs) in aqueous environmental samples as part of NOAA-CICEET funded research.

The development of the biosensor is a collaboration of Sapidyne Instruments, Inc (Boise, ID) that provided the KinExA Inline Sensor instrument and the Virginia Institute of Marine Science that developed a monoclonal antibody to 3-5 ring PAHs¹. In laboratory studies, the biosensor quantitatively measures 3-5 ring PAHs in water to ~0.3µg/L. Each sample takes ~10 minutes to analyze.

The goal of the study is to evaluate the biosensor's field performance under a variety of field conditions and venues. In collaboration with the US Fish & Wildlife Service, US EPA, Key Environmental Inc. and with the permission of the landowner, we accessed and sampled wells on the US EPA Superfund site LA Clarke & Son located in Spotsylvania County, VA on 25 March 2009. The LA Clarke & Son site is a former wood treating facility which used creosote and coal tar to preserve wood products. As a result of 50 years of processing, the site is contaminated with PAHs, benzene, and dense non-aqueous phase liquids.

Methods

Field site set-up –Using available onsite electricity, the biosensor was setup in a non-temperature controlled worksite shed. Some of the pumps and fittings of the biosensor leaked because of the less than optimal temperatures (~40°F). The biosensor was relocated to the heated truck which solved the problems.

Sample collection –James Zubrow of Key Environmental provided the results from previous analyses and combined with his knowledge of the site, 8 wells were selected for sampling. We selected wells ranging from low contamination to highly contaminated, in order to provide a wide range of PAH concentrations. Each well was pumped with enough water to purge the pumps and lines and then sampled. The samples collected were a 30ml amber vial for biosensor, then one 4L amber bottle for gas chromatography-mass spectrometry (GC-MS) analysis, followed by two 1L bottles for Key Environmental; all in precleaned containers. The samples collected for the biosensor were assessed as soon as possible (usually within 30 minutes), while the samples for GC-MS analysis were kept on ice until we returned to the lab.

Biosensor analysis – The sensitivity of the biosensor is such that all concentrations in excess of 10 μ g/L exceed the calibration curve; therefore these samples must be diluted in order to produce a quantifiable signal. Except for the dilutions, when necessary, samples were analyzed directly on the biosensor with no manipulation (i.e., no extraction or filtration).

Analytical analysis – For the GC-MS analysis, 1L of water was spiked with surrogate standards, extracted with three aliquots of dichloromethane solvent. Then the volume was reduced and internal standards were added prior to analysis using a GC-MS in electron ionization mode. The samples were analyzed for a wide range of PAHs as well as benzene, alkylated benzenes and heterocyclic aromatic hydrocarbons.

Results

The field site and well locations are shown in Fig 1. The sampling order of the wells was based on prior results from Key Environmental. The sampling order was from least to most contaminated starting with W7, W5, MW81, D8, PW01, D3, W6, and then MW80. Samples collected for the biosensor assessment were transferred to the biosensor upon collection.

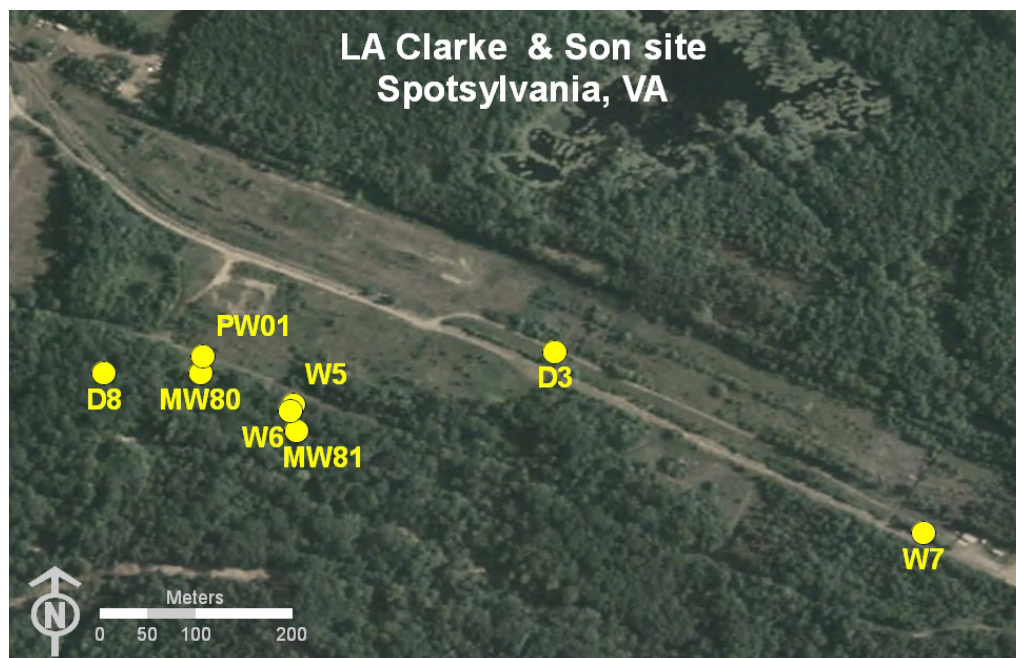


Figure 1. Location of the wells at the LA Clarke & Son site in Spotsylvania, VA sampled on 25 March 2009. The well locations and their names are designated by yellow dots.

Well samples W7, W5, MW81 and D8 did not require dilution for biosensor analysis. The samples from PW01, D3, W6 and MW80 were diluted before concentrations were determined.

The PAH concentrations from the biosensor and GC-MS analyses for each well are shown in Fig 2. The GC-MS results are presented as 1) total PAHs (including many of the compounds identified in the water soluble fraction of creosote) and as 2) a sum of 6 PAHs (phenanthrene, anthracene, fluorene, chrysene, pyrene, and fluoranthrene). The reason for the two forms of analysis are that the antibody has greater specificity for the 3-5 ring PAHs, so to serve as a proxy concentration we have selected the six most environmentally common 3 and 4 ring PAHs and summed their concentrations.

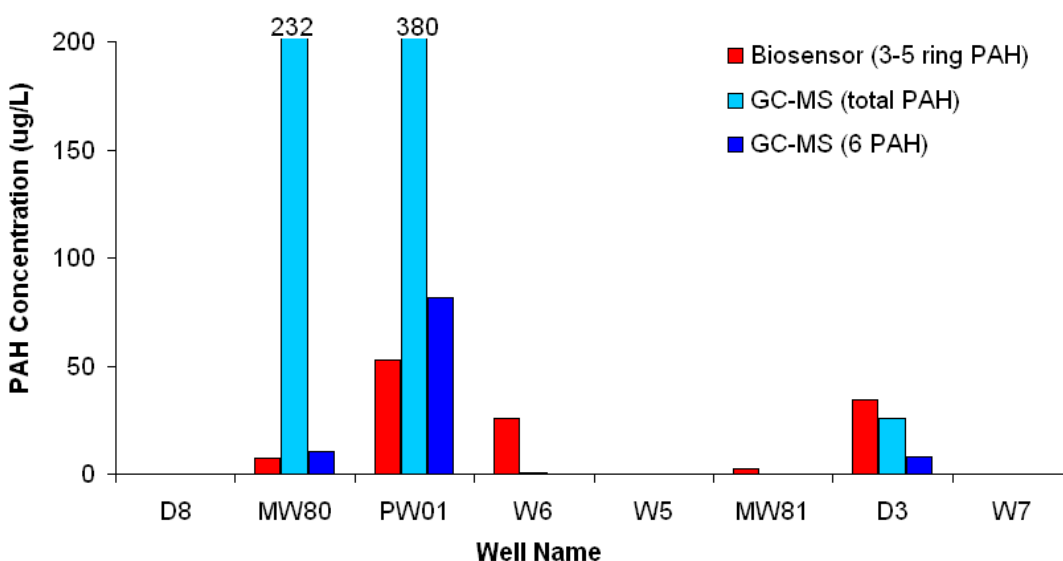


Figure 2. PAH concentrations ($\mu\text{g/L}$) for various wells at the LA Clarke & Son site in Spotsylvania, VA sampled on 25 March 2009. The red bars are the biosensor results, the light blue are the GC-MS results for total PAHs and the dark blue bars are the GC-MS sum of the 6 PAHs (phenanthrene, anthracene, fluorene, chrysene, pyrene, and fluoranthrene).

Well samples W7, W5 and D8 were below the detection limit of the biosensor and the GC-MS analyses confirmed these results, total PAHs ($<0.02\mu\text{g/L}$). The remaining wells had detectable concentrations of PAHs. For samples from wells MW80 and PW01, the biosensor-determined concentrations corresponded well with the sum of the selected 6 PAHs rather than the total PAHs, which is expected given the antibody's specificity for 3-5 ring PAHs. This relationship is evident in the case of well MW80, where much of the total PAH concentrations are a result of large contributions of the lighter 2 ring PAHs, indane and naphthalene. In the sample from well D3, the biosensor over estimates the PAH concentration, because the sample was mainly comprised of the larger 4 and 5 ring PAHs. The predominance of these compounds skewed the biosensor results in relation to the GC-MS results. Therefore the biosensor result correlates better with the total PAH sum and less with the 6 PAH sum

Conclusions and Discussion

In this field application we demonstrated the utility of the biosensor as a deployable field instrument providing near real time analyses. By assessing samples of varying PAH concentrations, we showed that the biosensor can assess environmental water samples without

any manipulations provided the concentrations are below 10µg/L. This also assumes that there was no impact on the biosensor from matrix affects for these samples. For samples above 10µg/L, dilutions in deionized water were an acceptable method to produce a signal within the biosensor's range of quantitation. Wells MW80 and PW01 substantiate our decision for selecting the 6 PAHs as an equivalent concentration to the biosensor's estimates. Although well D3 does not correlate well with the 6 PAH sum calculated from GC-MS data, the unusual predominance of 4 and 5 ring PAHs in this sample explains this discrepancy.

The antibody currently being used in this biosensor is one that is selective for 3-5 ring PAHs¹. Additional antibodies selective to 2-ring and alkylated PAHs would provide a better estimation of total PAH in the environment. An antibody to alkylated PAHs would help serve as a tool for determining the source material of a contaminant (i.e., oil vs. creosote or petrogenic vs. pyrogenic). Development of these antibodies are underway in our laboratory and once available, reassessment of this site would provide us with a great opportunity to expand our biosensor's capabilities.

Because of the biosensor's low limit of detection (<10 ppb), the biosensor is an ideal platform for rapid on-site analysis of low-level PAH contamination in environmental samples. This technology could easily be used to assess changes in PAH concentration in bodies of water and potentially locate its sources. Then targeted sampling of the site and traditional analytical methods can be used for more costly and time consuming GC-MS conformational analysis.

Acknowledgments

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References

1. Spier, C. R., Bromage, E. S., Harris, T. M., Unger, M. A., & Kaattari, S. L. (2009). The development and evaluation of monoclonal antibodies for the detection of polycyclic aromatic hydrocarbons. *Analytical Biochemistry*, 387(2), 287-293.