

XENOBIOTIC DETECTION SYSTEMS, INC.

Presents

The XDS-CALUX[®] Assay



Rapid, Accurate, and Affordable Dioxin/Furan and PCB Analysis

Mission Statement:

XDS exists to develop and advance the commercial acceptance of biological methods that will facilitate assessment of environmental and human health risks.

A Message from Our President...

On behalf of myself and the staff at Xenobiotic Detection Systems, Inc. I would like to take this opportunity to thank you for expressing interest in our company and its products. Our primary goal is to use the latest advances in biotechnology to develop accurate and cost-effective assays for environmental testing. This is becoming increasingly important as both government and industry realize that frequent testing is necessary to safeguard the environment and human health.

XDS is proud to offer you the patented **C**hemical **A**ctivated **L**uciferase Gene **E**xpression (CALUX[®]) assay. The XDS-CALUX[®] assay is the culmination of over 30 years of research in biotechnology and environmental testing. The result is a test for dioxins and dioxin-like compounds that offers fast results and superior accuracy at only a fraction of the cost of standard high resolution mass spectrometry analysis.

XDS is committed to providing quality analysis and developing biological assays to their fullest potential. I am confident that our testing methods will meet your expectations. We look forward to fulfilling your testing needs.

Sincerely,

George Clark, Dr. P.H.
President

George Clark brings over 30 years of experience to XDS. His education includes a Ph.D. in toxicology from the University of North Carolina at Chapel Hill and Masters degrees in immunology from the University of California at Berkeley and biochemistry from North Carolina State University. He has previously worked for GlaxoSmithKline, and the National Institutes of Health.



I. What can the XDS-CALUX[®] Assay Measure?

Persistent Bioaccumulating Toxins (PBT)

PBTs are chemicals that remain in the environment for long periods of time and accumulate in living organisms. Repeated low-level exposure to these chemicals result in toxic effects. Toxic effects are the result of the group's ability to alter normal biological processes. Substances classified in this group include PCDH, mercury, and polycyclic aromatic compounds.

The EPA has proposed new reporting requirements with lower thresholds for PBT. As a result of these changes, industries that produce PBT will be forced to increase their reporting. XDS has developed a low cost, accurate screening assay to meet these needs. The XDS-CALUX[®] assay has the potential to detect any of the compounds within this group.

Polychlorinated Diaromatic Hydrocarbons (PCDH)

PCDH are a subset of PBT. This subset includes dioxins and dioxin-like substances such as PCB and furans.

XDS has developed a dioxin specific XDS-CALUX[®] assay that can measure dioxins, PCB or furans. This dioxin specific assay can determine total concentration of all dioxins and dioxin-like compounds, or it can determine

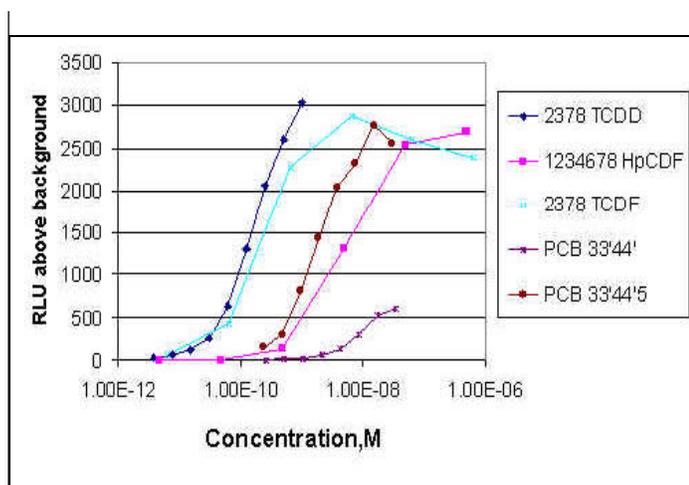


Figure 1: Dose response curves of the XDS-CALUX[®] assay to various PCDH

the concentrations of a specific class of compounds in a sample. Figure 1 shows luciferase response to several dioxins, furans and PCB congeners at various concentration levels.

Our Separation Guarantees Accuracy

In order to accurately measure low-level contaminants like dioxins, separation methods are critical. XDS has developed and patented (US 6,720,431 B2) methods that allow us to accurately separate PCDH, PCB, dioxins, furans, and other biological toxicants. The new separation methods combined with the XDS-CALUX[®] assay enable us to measure the total biological response of either specific classes of PCDH or total PCDH.

II. Uses

The XDS-CALUX[®] Assay is ideal for use as a low cost research tool to determine biological activity of a specific substance. It can also be used as a screening assay to measure contamination levels at industrial sites. Once contamination has been determined using the XDS-CALUX[®] assay, the identity of contaminants can be determined using techniques like GC-MS. The XDS-CALUX[®] assay saves customers money by decreasing the number of GC-MS samples needed, which are higher in cost than XDS-CALUX[®].

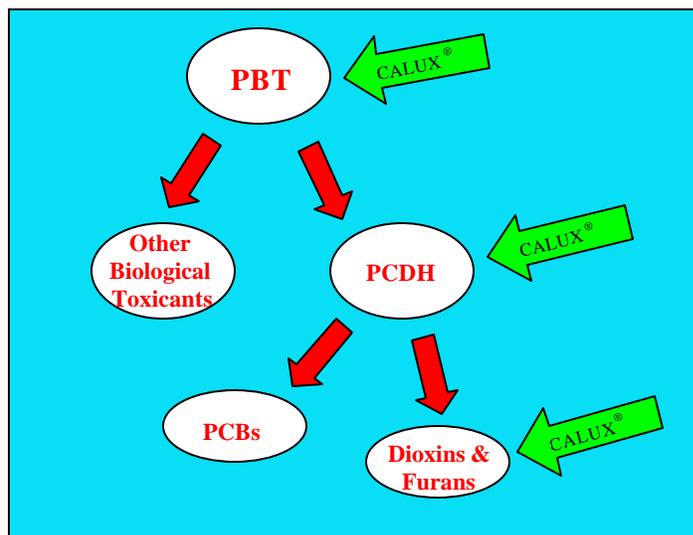


Figure 2: Diagram of XDS's separation scheme and the activity

III. Summary of Research

Background information

The effects of polychlorinated diaromatic hydrocarbons (PCDH) have been widely studied. PCDH include dioxins and dioxin-like compounds such as 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD), PCB and furans. PCDH have been known to accumulate in animals and cause species- and tissue-specific toxic effects. Birth defects, immune system disorders, tumor production, and death have all been observed as a result of high-level exposure to PCDH.



Mechanism of Action

The mechanism of action for these compounds depends on their ability to bind to an intracellular receptor called the aromatic hydrocarbon receptor (AhR). The PCDH-Ah Receptor complex then travels to the nucleus of the cell and binds to specific sequences in DNA called dioxin

Sensitive and Accurate

In a study by Garrison *et al.*, the firefly luciferase gene used by XDS-CALUX[®] has been shown to activate dioxins in a time, dose and AhR dependent manner. XDS routinely measures dioxins below one part per trillion concentrations by XDS-CALUX[®].

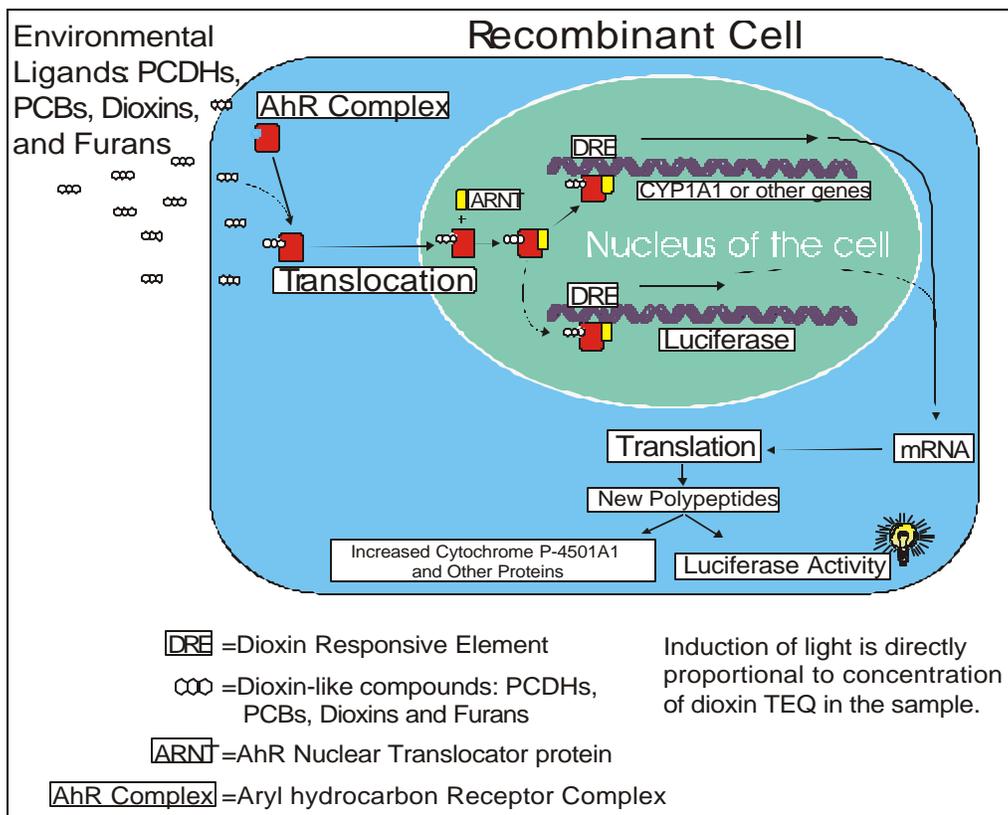


Figure 3: CALUX[®] cell mechanism

responsive elements (DRE). Binding of the PCDH-Ah Receptor complex to a DRE causes expression of the associated gene to be altered. It is this alteration in gene expression that causes observed toxic effects.

The XDS Chemical Activated Luciferase Expression Assay (XDS-CALUX[®]) applies the knowledge of the toxic mechanisms of PCDH. Using recombinant technology, the gene for firefly luciferase has been placed under the control of the Ah Receptor and inserted into a mouse cell line. Using this cell line, the presence of PCDH in a sample can be identified because PCDH will bind to the Ah Receptor, which will then bind to DRE in the nucleus and cause expression of firefly luciferase. The amount of PCDH in the sample can be related to how much light is produced by the activated cells.

Flexible and Versatile

The XDS-CALUX[®] bioassay was designed to detect biological and environmental toxicants in any medium. Studies have shown that XDS-CALUX[®] has accurately detected the concentration of dioxins in a number of matrices. Figure 4 (following page) illustrates the versatility of XDS-CALUX[®] and provides a sample of the various samples analyzed. The figure also shows the close correlation with HR GC/MS results.

International Reputation

XDS' CALUX[®] technology has been selected by the Belgian and Polish Governments as a screening tool for protecting their food supply and has been certified and accepted by the European Union as a valid dioxin detection method. Additionally, XDS has licensed and provided training to the US FDA and the Hiyoshi Corporation of Japan to use the patented XDS-CALUX[®] technology. XDS-CALUX[®] has also been accepted as the official bio-detection system for Dioxins/Furans in Japan.



IV. Pricing

The price of XDS-CALUX[®] analyses are 40% -70% lower than HR GC/MS fees. These substantial savings make XDS-CALUX[®] an excellent screening tool and allow researchers, laboratories, and government agencies the option to use expensive HR GC/MS analyses only when XDS-CALUX[®] results indicate dioxin levels are above client-established threshold levels. Savings exist even above 50% HR GC/MS follow-ups. Prices for analyses are determined based on the quantity and type of samples ordered, and the time requested to deliver results. Please email (info@dioxins.com) or call us (1-888-DIOXINS) for a written estimate. We offer order and volume discounts.

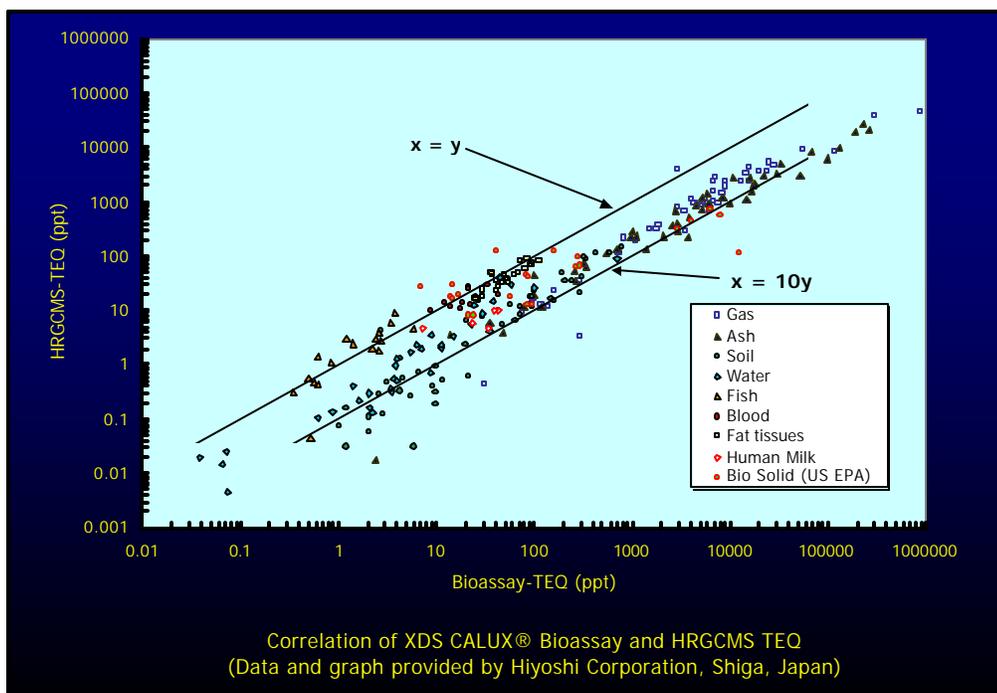
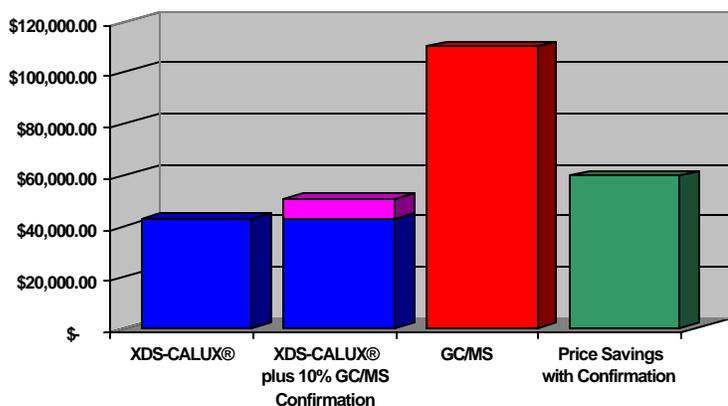


Figure 4: Comparison of XDS -CALUX[®] to HR GC/MS

Costs and Savings For XDS-CALUX[®] v.s. GC/MS

Note: Based on 100 samples with a \$1100 per sample rate for GC/MS and \$425 per sample rate for XDS-CALUX[®]. Neither XDS-CALUX[®] nor GC/MS pricing reflects volume discounts.



For additional information and research abstracts please contact Xenobiotic Detection Systems, Inc. at www.dioxins.com or (919) 688-4804.

V. Selected References

- Garrison, P.M. *et al.*, (1996). Species-Specific Recombinant Cell Lines as Bioassay Systems for the Detection of 2,3,7,8-Tetrachlorodibenzo-p-dioxin-like Chemicals. *Fundamental and Applied Toxicology*. 30, 194-203.
- Denison, M. S., Clark, G., *et al.*, (1996). Application of the CALUX[®] Bioassay System for the Detection of Dioxin-Like Chemicals (Ah receptors) in Whole Serum Samples and in Extracts From Chemical and Consumer Products. *Organohalogen Compounds*. 27, 280-284.
- Van Overmeire., *et al.*, (1999). A Comparative Study of GC-HRMS and CALUX[™] TEQ Determinations in Food Samples by the Belgium Federal Ministries of Public Health and Agriculture. *Organohalogen Compounds*. 57, 196-199.
- Clark, G., *et al.*, (1999). A Novel Low-Cost Air Sampling Device (AmbStack Sampler) and Detection System (CALUX Bioassay) for Measuring Air Emissions of Dioxin, Furan, and PCB on a TEQ Basis Tested With a Model Industrial Boiler. *Organohalogen Compounds*. 42, 309-312.
- Clark, G., *et al.*, (2001). Comparison of CALUX[®] Bioassay TEQ Determinations with High Resolution GC/MS Analyzing Soil Samples from a Hazardous Waste Site. United States - Vietnam Scientific Conference on Human Health and Environmental Effects of Agent Orange/Dioxins, March 3-6, 2002, Hanoi.
- Kayama, F. *et al.*, (2001). CALUX[™] Assay Is Applicable to Human Epidemiological Studies. *Organohalogen Compounds*. 54, 48-50.