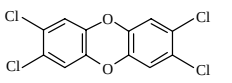
Detection of Halogenated Dioxins and Related Chemicals: Development, Validation and Application of a Novel Dioxin Cell Bioassay for Site and Sample Characterization

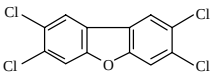
Michael S. Denison[1], Michael Ziccardi[1], David Baston[1], George C. Clark[2], David J. Brown[2], Michael Chu[2] and Hiroshi Murata[3]

[1]Department of Environmental Toxicology, Meyer Hall, University of California, Davis, CA, USA
[2]Xenobiotic Detection Systems, Inc., 1601 E. Geer Street, Suite S., Durham, NC, USA
[3]Hiyoshi Corporation, 908 Kitanoshocho, Omihachiman, Shiga, Japan.

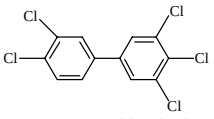
Halogenated Aromatic Hydrocarbons



2,3,7,8-Tetrachlorodibenzo-p-dioxin



2,3,7,8-Tetrachlorodibenzofuran

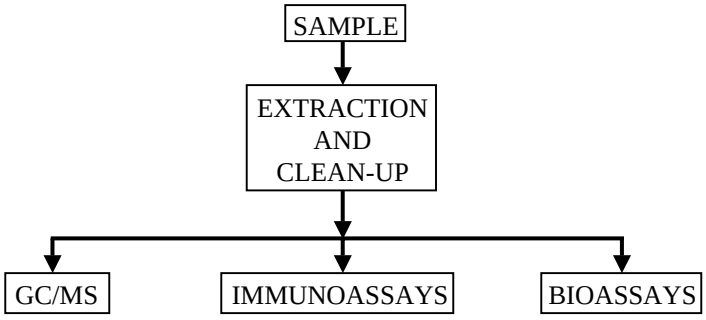


3,4,3',4,'5-Pentachlorobiphenyl

Spectrum of Toxic and Biological Effects Produced by TCDD in Different Species and Tissues

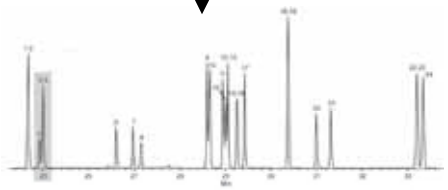
- Immunotoxicity
- Hepatotoxicity
- Wasting Syndrome
- Dermal Toxicity
- Teratogenicity
- Lethality
- Endocrine Disruption
- Tumor Promotion
- Porphyria
- Induction of Gene Expression (Also Repression)
  - Cytochrome P4501A1/2 and 1B1
  - Glutathione S-Transferase
  - UDP-Glucuronosyl Transferase 1*6
  - Quinone Reductase

Overview of Chemical and Biological Techniques to Detect and Quantitate Halogenated Dioxins and Related Chemicals



Biological/Toxic Potency Estimates of a Complex Mixture are Based on the Relative Potency of the Specific Compounds Present in a Given Complex Mixture

Sample Analysis by High Resolution GC/MS

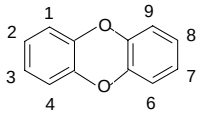
Environmental and Biological Samples → **Extraction and Clean-up Procedures** → **Halogenated Aromatic Hydrocarbons (HAHs)** → **High Resolution Gas Chromatography Mass Spectrometry** → 

Allows Determination of PCDD and PCDF Concentrations

But What About the Overall Biological/Toxicological Potency of the sample?

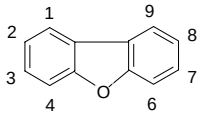
Halogenated Aromatic Hydrocarbon AhR Ligands
Toxic Equivalency Factors (TEFs)

PCDDs



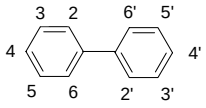
Congeners	TEF
2,3,7,8-TCDD	1.0
1,2,3,7,8-PeCDD	1.0
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.0001

PCDFs



Congeners	TEF
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.0001

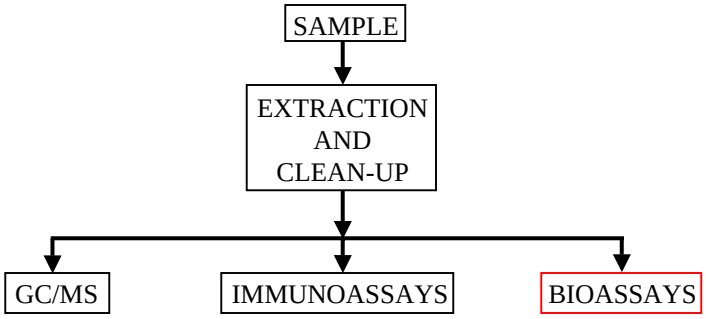
PCBs



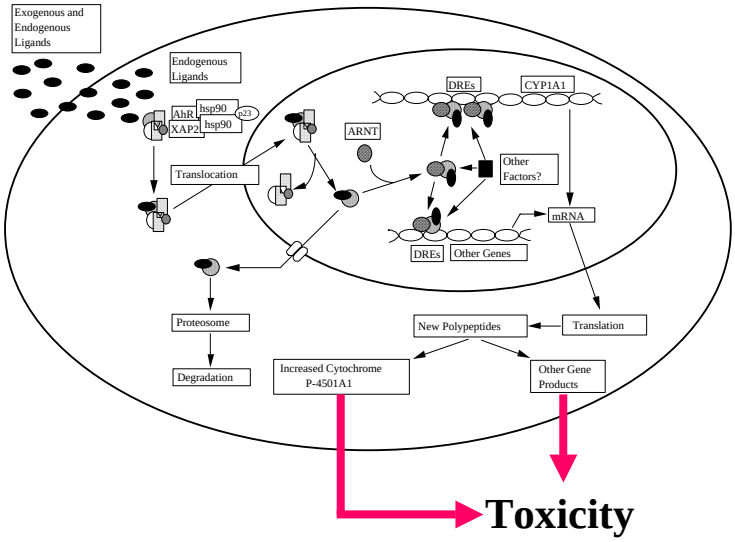
Congeners	TEF
3,3',4,4'-TCB	0.0001
3,4,4',5-TCB	0.0001
3,3',4,4',5-PeCB	0.1
3,3',4,4',5,5'-HxCB	0.01

$$TEQ = ([PCDD_i \times TEF_i]_n) + ([PCDF_i \times TEF_i]_n) + ([PCB_i \times TEF_i]_n)...$$

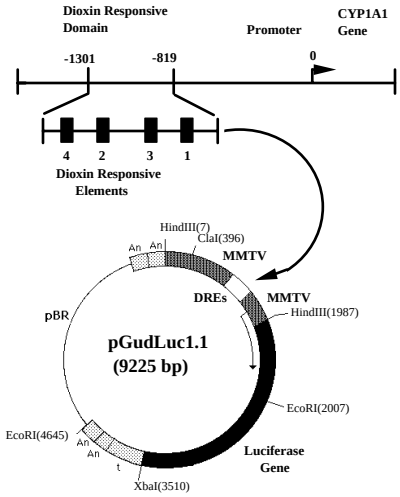
Overview of Chemical and Biological Techniques to Detect and Quantitate Halogenated Dioxins and Related Chemicals



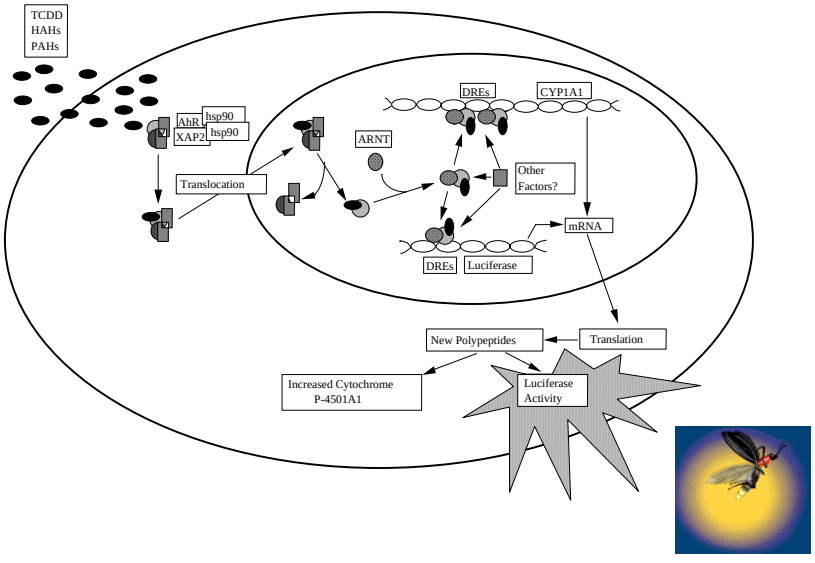
Action of Dioxin-Like HAHs Occurs Via A Common Mechanism: The Ah Receptor (AhR) Signal Transduction Pathway



Construction of a TCDD-Responsive Luciferase Reporter Gene Plasmid



CALUX (Chemically-Activated Luciferase Expression) Cell Bioassay

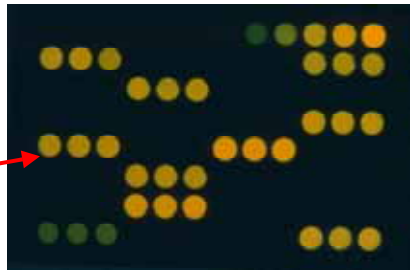


CALUX Bioassay Procedure

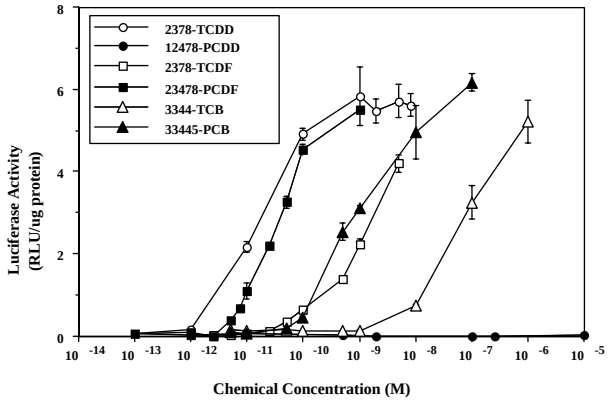
H1L6.1c3 Mouse Hepatoma Cells Plated into 96-Well Microplates

Chemicals Added to Each Well and Incubated for 24 hours

Wells are Washed, Cells Lysed, and Luciferase Activity Measured in a Microplate Luminometer



Activation of the CALUX Cell Bioassay by PCDDs, PCDFs and PCBs



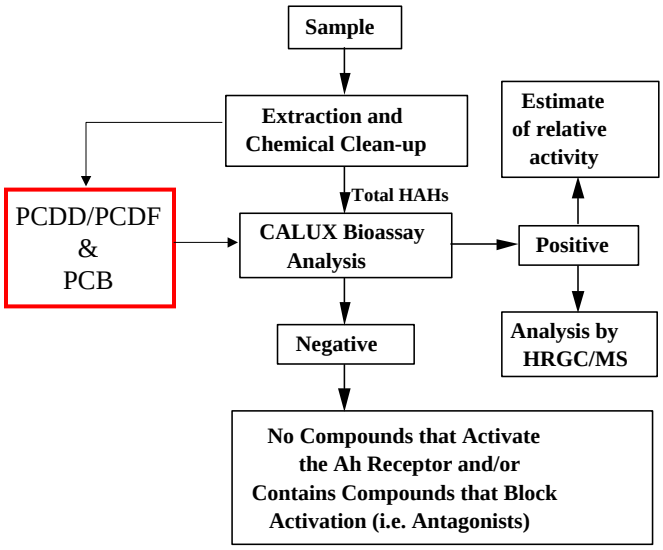
Assay Characteristics of TCDD in the CALUX Bioassay

EC50 = 10 pM, MDL = 1 pM ($MDL_{microplate}$ = ~100 ppq)
Dynamic Range = 2-3 Orders of Magnitude

WHO Toxic Equivalency Factors (TEFs) and CALUX Relative Potency (REP) Factors for Chlorinated Dibenzo-p-Dioxins, Dibenzofurans and Biphenyls.

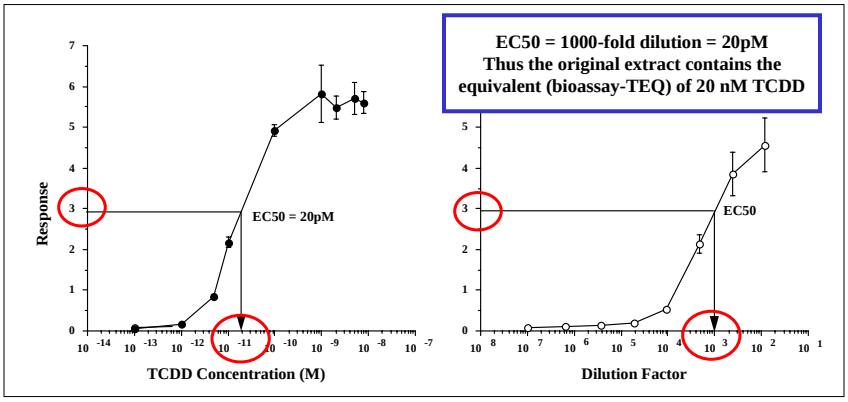
Compound	WHO-TEF	CALUX REP
2378-TCDD	1	1.00 ± 0.01
12378-PeCDD	1	0.73 ± 0.1
123478-HxCDD	0.1	0.075 ± 0.014
123678-HxCDD	0.1	0.098 ± 0.017
123789-HxCDD	0.1	0.061 ± 0.012
1234678-HpCDD	0.01	0.031 ± 0.008
OCDD	0.0001	0.00034 ±0.00008
2378-TCDF	0.1	0.67 ± 0.01
12378-PeCDF	0.05	0.14 ± 0.04
23478-PeCDF	0.5	0.58 ± 0.08
123478-HxCDF	0.1	0.13 ± 0.02
123678-HxCDF	0.1	0.14 ± 0.03
123789-HxCDF	0.1	0.11 ± 0.02
234678-HxCDF	0.1	0.31 ± 0.06
1234678-HpCDF	0.01	0.024 ± 0.007
1234789-HpCDF	0.01	0.044 ± 0.010
OCDF	0.0001	0.0016 ± 0.0005
PCB 77	0.0005	0.0014 ± 0.0004
PCB 81	0.0001	0.0045 ± 0.0012
PCB 114	0.0005	0.00014 ± 0.00002
PCB 126	0.1	0.038 ± 0.007
PCB 156	0.0005	0.00014 ± 0.00002
PCB169	0.01	0.0011 ± 0.0003

Flow Diagram for Analysis of Samples by CALUX and GC/MS



CALUX Cell Bioassay - Considerations

• Calculation of relative biological potency of an unknown sample extract



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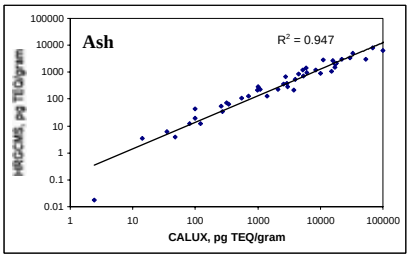
Applications of the CALUX Dioxin Cell Bioassay

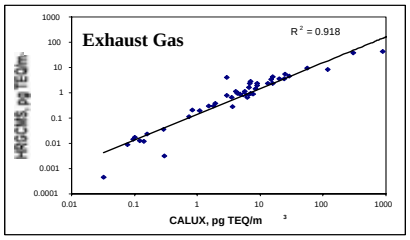
1. Biological Samples
   - Blood (whole serum and extracts)
   - Breast Milk
   - Tissue Extracts

2. Environmental Samples
   - Soil and Sediment
   - Ash
   - Emission (PUF)
   - Pulp and Paper

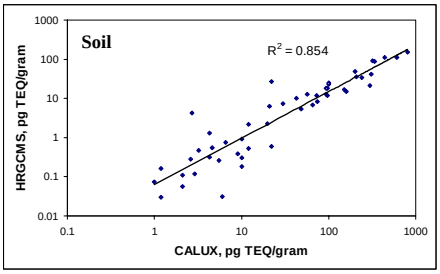
3. Food Samples
   - Animal Fats (oil and fats)
   - Milk and Butter
   - Animal Feeds

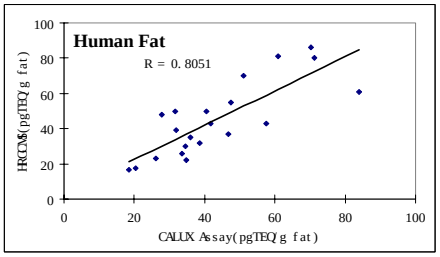
Double-Blind Validation Results Comparison of CALUX and HR GC/MS Analysis of Ash and Exhaust Gas Samples



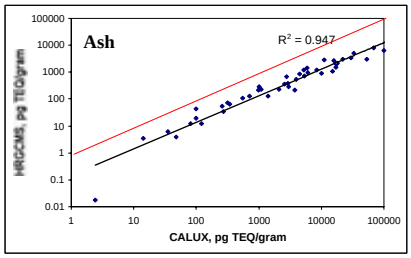


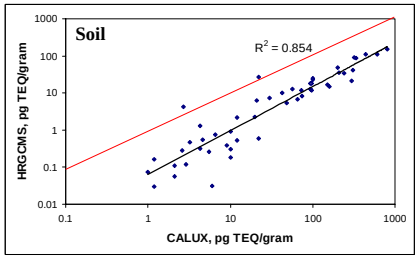
Double-Blind Validation Results Comparison of CALUX and HR GC/MS Analysis of Soil and Human Fat Samples

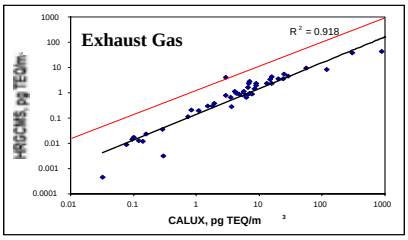


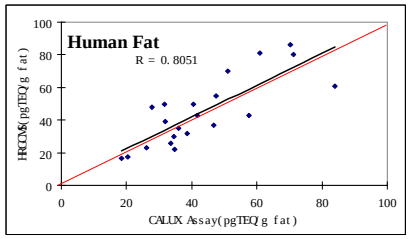


Environmental Samples Contain Additional Ah Receptor Active HAHs



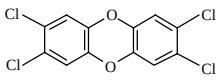




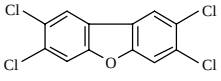


One Possibility for the higher equivalent estimates: PB/CDDs/Fs

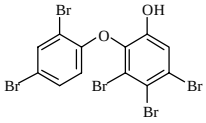
Halogenated Aromatic Hydrocarbons



2,3,7,8-Tetrachlorodibenzo-p-dioxin

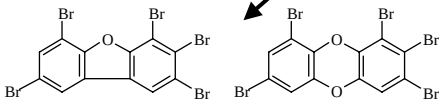


2,3,7,8-Tetrachlorodibenzofuran



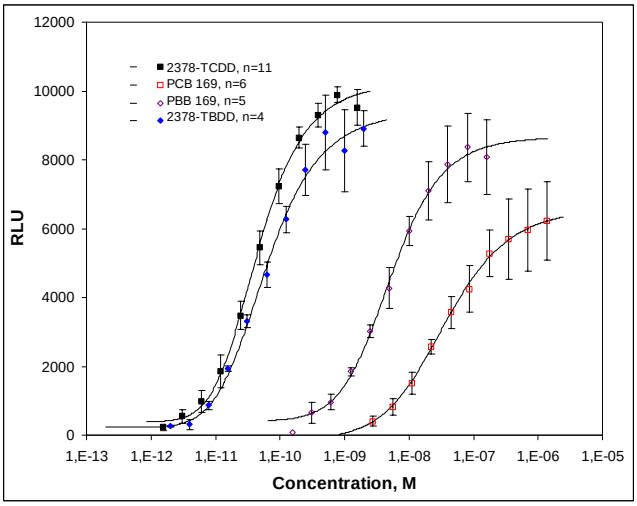
Polybrominated Diphenyl Ether

Sources:
Formed during PDBE Synthesis
Burning of PBDE Containing Materials
Photochemical Reactions of PBDEs



Polybrominated Dibenzofurans (PBDFs)
Polybrominated Dibenzo-p-dioxins (PBDDs)

PBDDs/Fs and PBBs are Active in the AhR-CALUX Bioassay.



AhR Gene Expression Cell Bioassay Systems

Advantages

- Sensitive, relatively rapid and easy to carry out.
- Amenable to high throughput analysis.
- Relatively inexpensive compared to instrumental analysis.
- A significant amount of validation data is available for the CALUX bioassay.
- SW846 Method Certification currently being compiled for USEPA submission.
- Overestimate TCDD equivalents in environmental samples (new dioxin-like HAHs?).

Disadvantages/Limitations

- Experience in cell culture techniques necessary (not difficult).
- Proper sample cleanup methods and methods to correct for extraction and clean-up efficiency and recovery are needed (can't spike with AhR-active HAHs like TCDD).
- Instrumentation - luminescent microplate readers, cell culture facilities.
- Overestimate TCDD equivalents in environmental samples (false positives?).
- Extracts containing chemicals that are directly toxic to cells can't be analyzed.
- Synergistic/antagonistic effects (over/underestimate potency - can be detected).

Application and Utilization of Bioanalytical Methods for Dioxin Analysis

- Detection, quantitation and chemical identification of dioxin-like chemicals in a variety of matrices including:
  - Environmental samples (soil, water, air)
  - Biological (blood, milk, tissues)
  - Food and feed
  - Commercial and consumer products
- Determination of the effectiveness of bioremediation, biodegradation and contamination clean-up procedures for dioxin-like chemicals.
- Identification and characterization of other classes of dioxin-like chemicals.

Acknowledgements and Support

University of California @ Davis
Joe Rogers, Patricia Garrison

Xenobiotic Detection Systems, Durham, NC
Mick Chu, Andrew Chu

Scientific Institute of Public Health, Brussels, Belgium
Leo Goeyens, Ilse van Overmeire

National Institutes of Environmental Health Sciences (NIEHS)
Superfund Basic Research Programs - ESO4699, ES04911