CALUX TESTING OF POPS CONTAMINATED SAMPLES
RESULTS FROM FIELD TESTING IN CAMBODIA, LAO PDR, MALAYSIA AND THAILAND
2009.07.24
Hiyoshi Corporation

Ⅰ About Hiyoshi
Ⅱ Principles of biomonitoring
Ⅲ Design principles of biomonitoring
Ⅳ Result from biomonitoring
Company Profile

Firm Name: Hiyoshi Corporation
Foundation: December 23, 1955
Sales: 4,700,000,000 yen (2007)
Employees: 266
President: Hiroshi Murata
Location: Headquater: 908 Kitanosho, Omihachiman, Shiga
Tokyo office: 9-1-17, Akasaka, Minatoku, Tokyo

80 Business Registrations and 1400 Individual Employees Licenses

Our business field

Measurement & Analysis
Facility Operation and Management
Urban Environment Management

We look “one step ahead” not as special business, but as a total environmental conservation business
Local Activity and International dedication
“International Trainees”

Every year we accept trainees from Asia and from other countries through public organizations to help them learn our new techniques in Ecological services.

Ⅰ About Hiyoshi
Ⅱ Principles of biomonitoring
Ⅲ Design principles of biomonitoring
Ⅳ Result from biomonitoring
Health Effects of Dioxin-Like HAHs

<table>
<thead>
<tr>
<th>PCDDs</th>
<th>PCDFs</th>
<th>PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="PCDDs" /></td>
<td><img src="image" alt="PCDFs" /></td>
<td><img src="image" alt="PCBs" /></td>
</tr>
</tbody>
</table>

**Toxicity**
- Cancer
- Immunotoxicity
- Heart disease
- Liver toxicity
- Skin toxicity
- Birth defects
- Wasting syndrome
- Lethality

**Biochemical**
- Endocrine disruption (estrogen/testosterone)
- Inhibit cancer cell growth
- Inhibit cell division
- Alter gene expression
- Drug metabolism enzymes increase

Sources of Dioxin-Like HAHs

- **Herbicide Spraying** (i.e. Agent Orange)
- **Combustion**
- **Pesticide (Chlorophenols)**
- **Transformers (PCBs)**
Mechanism of CALUX
Chemically-Activated Luciferase gene Expression

**Reporter Gene Assay**

Mouse hepatoma H1L6.1 cell line

Dioxins etc.

AhR : arylhydrocarbon receptor
Arnt : AhR nuclear tranclocator
DRE : dioxin responsive element
Hsp90: heat shock protein 90 kD

What are dioxin analysis?

Chemical Analysis
Chemical Agents
Chemical Reaction
Actual Quantity

Biological Analysis
Biological Material
Biological Reaction
Amount of Reaction

Target substances

Evaluate toxicity by biological reaction using living organism

HRGC/HRMS
CALUX(BIOASSAY)
CALUX use in the world

1998 Japan Hiyoshi Corporation start validation
2000 Japan Hiyoshi Corporation set up a laboratory
2003 Japan Ministry of the environment Established
2005 CALUX assay approved as an official method in Japan [flue gas, Ash]

1998 Patented in USA and Canada
2001 Adapted for dioxin analysis in food by US FDA
2002 USA EPA applied for Analysis of Biosolid
2007 USA EPA Method SW-846 [Solid Waste]

1999 Belgium government used as screening of dioxin contaminated in chicken
2000 Belgium SIPH set up a laboratory for dioxin screening analysis in food, feed, and biological samples
2002 Applied for food and feed regulation in EU (2002/69/EC) bioassay is adapted as screening method [Food sample]
2003 Belgium adapted Federal Feedings Laboratory
2005 Poland adapted National Veterinary Research Institute in Pulawy

Research on CALUX

CHINA:
2005 Give an presentation at China-Japan Joint Symposium on Environmental Chemistry
2005 Give a lecture on dioxin monitoring technique and training
2007-2008 Interns from Tsinghua Univ. (Soil & Sediment)

KOREA: 2005 Under review (by NIER)

LAOS: 2007 Dioxins and PCBs Monitoring in Lao PDR by World Bank in ERI (STEIA)

Taiwan: 2008 Training from National Health Research Institutes

VIETNAM:
2002 Vietnam-USA Scientific conference
2005 Research on 120 soil samples (XDS) in Vietnam
2005 Research on fish in Vietnam

THAI: 2007 Under review (by MOPH)

INDIA:
2006 Interns from SRM Deemed University
2006 Joint study under review
2007 Interns from IIT analyzed Indian spices and Milk products.
International Standard

1) Approved by Japanese Ministry of the Environment
   2005/9/14  Ministry of Environment, Notification 92
   Simple method (biological assay) can be officially used

2) EU 2002/69/EC
   Accepted biological assay for
   screening of food and feed regulation

3) USEPA Method 4435 (2005)
   applicable to solid waste

Application

Environmental samples

Food and Biological samples
Flowchart of CALUX

Characteristics of CALUX 1

Compact equipment has decreased amount of solvent use and shortened treatment time.
Characteristics of CALUX 2

Double serial column will be sufficient instead of complex HRGCMS process; multilayer silica gel – activated carbon and sometimes HPLC

Characteristics of CALUX 3
Characteristics of CALUX 4

**CALUX Assay 5 merits**

1. High Sensitivity
2. Less Expensive
3. Faster Turnaround
4. Simple Mechanism
5. Highly Efficient

<table>
<thead>
<tr>
<th>Cost</th>
<th>1/4</th>
<th>1/8</th>
</tr>
</thead>
</table>
| GC/MS      | $1300 | $300*
| CALUX      |      |     |

**Turn-around**

- 40 days
- 3-5 days*

*Varies from Sample types

- Concentration: 1pg-TEQ/g
- Sample Size: <10g

- Quantity of Organic Solvent: GC/MS 1/6 reduction
- Electric Consumption: GC/MS 1/20 reduction

The technology that emits less CO₂

= Environmental friendly technology

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I About Hiyoshi
II Principles of biomonitoring
III Design principles of biomonitoring
IV Result from biomonitoring
Purpose 1

1. Hatfield Consulting has carried out assessment of the risks to human health and the environment and comprehensive management of POPs and POPs-like compounds in the four countries.

2. In the context of above program, focused on monitoring of POPs and POPs-like compounds, among the 4 countries Lao PDR were selected as pilot country and CALUX was selected as monitoring tool.

Purpose 1

3. Hiyoshi has carried out the monitoring of POPs and POPs-like compound two times.

First time (2007): Soil 50 and Fish 6

Second time (2008): Soil 40, Fish 2 and Breast milk 36

Result of the monitoring project in 2007 has showed that CALUX is supplemental to traditional HRGC/HRMS method and simpler approach to POPs environmental analysis. Dioxin, furan and DL-PCBs were detected from various place and high altitude place, e.g. Nong Suin, and has showed that it is promising method for risk assessment of POPs contaminated site.
Purpose 2

4. In Workshop for the Regional Program held in Lao PDR in 2008 further use of CALUX was considered as effective method for the project. This also matches purpose of the project, capacity building. Hatfield consulting has identified “hotspot” as a part of Risk assessment project for Cambodia, Lao PDR, Malaysia and Thailand. Risk of exposure to contaminants from the site to human health will be assessed.

Hatfield Consultants will collect samples at each of the candidate hotspots and test them by HRGC/HRMS to determine risk effect to human health.

Purpose 3

5. Proposed follow-up project to establish CALUX method is as follow:
   a. Hatfield Consultants will collect two set of each soil, fish, and sediment (if applicable) samples from Cambodia, Lao PDR, Malaysia and Thailand.
   b. One set of samples will be tested by Hatfield using HR GC/HRMS.
   c. A second set of samples will be sent to Hiyoshi’s laboratory in Japan for CALUX analysis.

6. The proposed approach will aim following goal
   a. Strengthen the relationship between two analytical approaches for POPs monitoring in the context of site-specific contaminant characterization. In particular, it will further illustrate the usefulness of combining CALUX and HRGC/HRMS methods to monitor contamination of soil, fish or sediments.
Due to soil permission problem, some of the samples were sent to US via Canada and extracted samples were sent to Hiyoshi, Japan.

Based on the preliminary results by the bioassay significant samples were selected and tested using HRGC/HRMS by Canadian laboratory.
Summary

1. Selected Nation
   - Cambodia, Lao PDR, Malaysia, Thai land

2. Sampling Staff
   - HATFIELD CONSULTANTS and local experts

3. Target Samples/ Target Location
   - Cambodia: Soil 23, Fish 4, Water 1
   - Lao PDR: Soil 30
   - Malaysia: Soil & Sediment 13
   - Thai land: Soil & Sediment 30, Fish 5
Sampling point (Cambodia)

Result of CALUX (Cambodia)

From «Final Risk Assessment Report for Sambour EDC Warehouse Phnom Penh, Cambodia»
Result of HRGC/HRMS (Cambodia)

| Sampling point (Lao PDR) |

Fish Tissue Data:

| 08CAM029B Crab tissue | 0.207 | 0.243 | 0.015 | 0.0404 | 1.84 | 2020 |

"NM" indicates that the parameter was not assessed.

"TEQ" is toxic equivalence quotient.

1 Concentration was based on dry weight of soil.

2 Concentration was based on wet weight of tissue.

From 「Final Risk Assessment Report for Sambour EDC Warehouse Phnom Penh, Cambodia」

From 「Final Risk Assessment Report for Sok Pa Loung Case Study Site Vientiane, Lao PDR」
Result of CALUX (Lao PDR)

Result of HRGC/HRMS (Lao PDR)

From 「Final Risk Assessment Report for Sok Pa Loung Case Study Site Vientiane, Lao PDR」
Sampling point (Malaysia)

From «Final Risk Assessment Report for Air Hitam Sanitary Landfill Site Selangor, Malaysia»

Result of CALUX (Malaysia)
Result of HRGC/HRMS (Malaysia)

<table>
<thead>
<tr>
<th>Sediments</th>
<th>Location</th>
<th>PCDDs/Fs - TEQs (TEQs, WHO 2005)</th>
<th>PCDDs/Fs + DL-PCB (TEQs, WHO 2005)</th>
<th>Total PCDDs/Fs</th>
<th>Total PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pg TEQ/g HR-GCMS</td>
<td>pg TEQ/g HR-GCMS</td>
<td>p/q U</td>
<td>p/q U</td>
</tr>
<tr>
<td>08MAL009B</td>
<td>From treatment pond</td>
<td>ND=0: 14.2</td>
<td>ND=1: DL</td>
<td>ND=0: NM</td>
<td>ND=0: NM</td>
</tr>
<tr>
<td>08MAL010B</td>
<td>Residential area south of entrance</td>
<td>0.342</td>
<td>0.368</td>
<td>0.342</td>
<td>0.378</td>
</tr>
</tbody>
</table>

"ND" indicates that the concentration was below the method detection limit.
"NM" indicates that the parameter was not assessed.
"TEQ" is toxic equivalence quotient.

From "Final Risk Assessment Report for Air Hitam Sanitary Landfill Site Selangor, Malaysia"

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Sampling point (Thailand)

From "Final Risk Assessment Report for Case Study Site in Samut Prakan, Thailand"
Result of CALUX (Thailand)

**PCDDs/Fs, DL-PCBs, DXNs**

Sample No.

Result of HRGC/HRMS (Thailand)

<table>
<thead>
<tr>
<th>Soils or Dust</th>
<th>PCDDs/Fs - TEs (TEQs, WHO 2005)</th>
<th>DL-PCBs (TEQs, WHO 2005)</th>
<th>Total PCDDs/Fs</th>
<th>Total PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>08THA005</td>
<td>ND=0</td>
<td>ND=1/2DL</td>
<td>ND=0</td>
<td>ND=1/2DL</td>
</tr>
<tr>
<td>08THA010</td>
<td>Storage area (concrete floor)</td>
<td>197.00</td>
<td>199.00</td>
<td>NM</td>
</tr>
<tr>
<td>08THA019</td>
<td>Ditch near the storage area.</td>
<td>152</td>
<td>154</td>
<td>NM</td>
</tr>
<tr>
<td>08THA0419</td>
<td>River bed center of site.</td>
<td>1.59</td>
<td>1.61</td>
<td>386.44</td>
</tr>
<tr>
<td>Tissue Data</td>
<td>08THA-036</td>
<td>0.0532</td>
<td>0.094</td>
<td>NM</td>
</tr>
</tbody>
</table>

*NM* indicates that the parameter was not assessed.

From «Final Risk Assessment Report for Case Study Site in Samut Prakan, Thailand»
HRGC/HRMS vs CALUX 1

The result of CALUX and HRGC/HRMS analysis of the samples collected in this study

<table>
<thead>
<tr>
<th>Sample name</th>
<th>CALUX (TEQ, WHO2005)</th>
<th>HRGC/HRMS (TEQ, WHO2005 ND=1/2DL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCDD/Fs</td>
<td>DL-PCBs</td>
</tr>
<tr>
<td>08CAM010B</td>
<td>0.44</td>
<td>(2.1)</td>
</tr>
<tr>
<td>08CAM021B</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>08CAM022B</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>08LAO010B</td>
<td>72</td>
<td>25</td>
</tr>
<tr>
<td>08LAO016B</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>08LAO032B</td>
<td>66</td>
<td>253</td>
</tr>
<tr>
<td>08MAL009B</td>
<td>13</td>
<td>2.8</td>
</tr>
<tr>
<td>08MAL010B</td>
<td>0.63</td>
<td>(0.03)</td>
</tr>
<tr>
<td>08THA005B</td>
<td>14</td>
<td>217</td>
</tr>
<tr>
<td>08THA010B</td>
<td>9</td>
<td>171</td>
</tr>
<tr>
<td>08THA019B</td>
<td>0.98</td>
<td>(0.15)</td>
</tr>
<tr>
<td>08CAM029B</td>
<td>0.34</td>
<td>(0.38)</td>
</tr>
<tr>
<td>08THA-036B</td>
<td>0.41</td>
<td>(0.26)</td>
</tr>
</tbody>
</table>

Regional Capacity Building Program for Health Risk Management of Persistent Organic Pollutants (POPs) in South East Asia
Second Progress Report  FINAL  April 2009

HRGC/HRMS vs CALUX 2

![Graph showing the relationship between CALUX and HRGC/HRMS (TEQ, WHO2005) concentrations. The equation y = 1.2281x with R = 0.572 (n=26) is displayed.]
Since we noticed that the results of both PCDD/Fs and DL-PCBs were very different we have confirmed the samples. The Laos samples has taken multiple process; sent from Laos -> extracted at XDS -> cleanup and measured at Hiyoshi and this complicated process may have caused the inconsistent results. We have re-confirmed the stored extraction sent from XDS.

1)0010B, 2)0016B, 3)0032B (arrowed extraction samples) Color of the samples were darker than other samples which shows high possibility of high concentration.
HRGC/HRMS vs CALUX 4

- Lao PDR site
  - Linear regression: $y = 1.2281x$, $R = 0.572$ (n=26)

- PCDD/Fs + DL-PCBs
  - Linear regression: $y = 1.161x$, $R = 0.987$ (n=26)

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HRGC/HRMS vs CALUX 5

<table>
<thead>
<tr>
<th>Sample name</th>
<th>CALUX (TEQ,WHO2005)</th>
<th>HRGC/HRMS (TEQ,WHO2005 ND=1/2DL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCDD/Fs</td>
<td>DL-PCBs</td>
</tr>
<tr>
<td>08CAM010B</td>
<td>0.44</td>
<td>(2.1)</td>
</tr>
<tr>
<td>08CAM021B</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>08CAM022B</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>08LAO010B</td>
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<tr>
<td>08MAL009B</td>
<td>13</td>
<td>2.8</td>
</tr>
<tr>
<td>08MAL010B</td>
<td>0.63</td>
<td>0(&lt;1.0)</td>
</tr>
<tr>
<td>08THA005B</td>
<td>14</td>
<td>217</td>
</tr>
<tr>
<td>08THA010B</td>
<td>9</td>
<td>171</td>
</tr>
<tr>
<td>08THA019B</td>
<td>0.98</td>
<td>(1.5)</td>
</tr>
<tr>
<td>08CAM029B</td>
<td>0.34</td>
<td>(0&lt;0.38)</td>
</tr>
<tr>
<td>08THA-036B</td>
<td>0.41</td>
<td>0(&lt;0.26)</td>
</tr>
</tbody>
</table>

**BIOLOGICAL**

- Lao Project-1 (ENVIRONMENT) Soil
  - Bx1-2
    - PCDD/Fs: 30
    - DL-PCBs: 0
    - Total: 30
  - Bx1-19
    - PCDD/Fs: 0.62
    - DL-PCBs: 4.4
    - Total: 5.0
  - Bx2-2
    - PCDD/Fs: 1.3
    - DL-PCBs: 11
    - Total: 12
  - Bx2-8
    - PCDD/Fs: 45
    - DL-PCBs: 290
    - Total: 340
  - Bx3-3
    - PCDD/Fs: 38
    - DL-PCBs: 9
    - Total: 50

**ENVIRONMENT**
**Point**

The CALUX analytical results were used at three major steps of the risk assessment process for all four case study sites, as follows:

* Prescreening of samples
  The CALUX analytical results were used to guide selection of environmental samples for HRGC/HRMS analysis

* Screening of contaminant level
  For PCDD/Fs (i.e., dioxins/furans) and PCBs, the CALUX TEQ concentrations results were used for screening of environmental samples to determine if a chemical is present at potentially hazardous concentrations against environmental quality guidelines; and

* Modeling input for risk calculation
  The results of the CALUX and the HRGC/HRMS analysis were both considered for selecting model input concentrations, which is consistent with the conservative approach adopted for screening level risk assessments. The higher of the CALUX or HRGC/HRMS results was adopted for the risk assessment calculations. Only three analytical results by HR GCMS were found slightly higher than those from similar results analyzed by CALUX.
Case of applying the screening method by Ministry of the Environment Japan

Manual on Determination of Dioxins in Soil has established in 2009 and now HRGC/HRMS both can be used for soil contamination research.

• Procedure when contaminated area cannot be estimated: In such case, collect samples from equal distance from contaminated site. Continue to expand the area until sample level fulfill pollution limit.

Flow chart proposal for Dioxin research in Developing countries

HRGC/HRMS Possessing country

- Exceeded the limit
- Detail research by HRGC/HRMS

CALUX installed country (developing country)

- Research on land history
- Research on generation and surrounding condition
- Determine sampling method (place and number)
- Implement soil research by CALUX
- Convert and estimate HRGC/HRMS result using conversion factor

- Determination limit
- Less than the limit
- No further research necessary
- Get result and consider measurement
Introduction of Easier Sampling Method (exhaust)

Introduction of Easier Sampling Method (water)

Sample water → Add reagent and mix → Leave it to settle → Vacuum filtration → Soxlet extraction
Conclusion 1

• Effectivity of CALUX has been already approved in US, EU and in Japan and is being used at various laboratory.
• Through the World Bank Project, we were able to confirm effectivity of CALUX in East Asia (especially for PCB contaminated region).

Following can be done:
1-1) Samples (collected and extracted) can be analyzed in Japan. (Contract analytical survey)

1-2) UNEP toolkit dioxin inventory estimation can be evaluated by doing actual analysis (Research on POPs measurement)
Conclusion 2

2) Establish dioxin laboratory in East Asia (installation of CALUX laboratory) will be possible.

As the first step:
Establish model laboratory and implement dioxin survey around East Asian country to diffuse and evaluate the technique.

We hope to support establishing model laboratory with our experience of more than 10 years in training interns and technical staffs from abroad.

Thank you for your attention

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http://www.dioxins.com/