Development and Use of Screening Methods for Rapid Characterization

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FIRST GENERATION OF IMMUNOASSAY METHODS IN SW-846

• First group of immunoassay methods formally added to EPA OSW methods manual, SW-846, in June, 1997.

• One generic method describing the ELISA technique (Method 4000).
FIRST GENERATION METHODS

continued

• Ten screening methods for individual compounds (PCP, 2,4-D, DDT, Toxaphene, Chlordane, TNT, RDX) or compound classes (PCBs, TPH, PAHs).

• Immunoassay screening method development guidance document.

• Approx. 26 validated kits from 4 original manufacturers
## Approved Immunoassay Methods

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<thead>
<tr>
<th>Method Analyte</th>
<th>Manufacturer</th>
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<tr>
<td></td>
<td>A</td>
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<tr>
<td>4010 PCP</td>
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<td>4015 2,3-D</td>
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<td>4020 PCB</td>
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<td>4025 Dioxins</td>
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<td>4030 TPH</td>
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<td>4035 PAH</td>
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<tr>
<td>A</td>
<td>B</td>
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<tr>
<td>4040 Toxaphene</td>
<td>Soil</td>
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<tr>
<td>4042 DDT</td>
<td>Soil</td>
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<tr>
<td>4050 TNT</td>
<td>Soil</td>
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<tr>
<td>4051 RDX</td>
<td>Soil</td>
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<tr>
<td>4500 Mercury</td>
<td>Soil</td>
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<tr>
<td>4670 Triazines</td>
<td>Water</td>
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Approved Immunoassay Methods Manufacturers

- A - BioNebraska
- B - Cape Technology
- C - Beacon (SDI)
- D - EnSys (SDI)
- E - Millipore (SDI)
- F - Ohmicron (SDI)
- G - SDI
NEW DEVELOPMENTS IN ELISA SCREENING METHODS

• Beacon Analytical kits for PAHs and PCBs.

  – First generation PAH kits sensitive to either 2 to 4 ring PAHs (phenanthrene) or 5 to 7 ring compounds (benzo(a) pyrene)

  – New Beacon kit targets 4-membered rings and covers the entire range of PAHs in one kit
NEW DEVELOPMENTS continued

– New PCB kit covers wider range of PCBs including greater sensitivity to lower chlorinated congeners with sharper response than first generation kits.
IMMUNOSENSORS

• Two new methods for TNT and RDX explosives using existing ELISA antibodies and colorimetric detectors (NRL).
  – Flow cell detector (Method 4655).
  – Fiberoptic detector (Method 4656).
IMMUNOSENSORS continued

• Sensitivity of 10 ppb in water for explosives.

• Soil methods nearing completion.

• Projects planned to expand applicability of immunosensor technology to additional analytes, e.g., PAHs, PCBs, TCE
GROSS SCREENING METHOD USING A REPORTER GENE

• New method for planar organic compounds, PAHs, PCBs, PCDDs/PCDFs using a reporter gene on a human cell line, (Method 4425) (Columbia Analytical Services).

• Cytochrome P-450 group of enzymes and human liver cell line.
GROSS SCREENING METHOD
continued

- Method can differentiate between PCBs, PAHs and dioxin/furans on a site by differences in development times, but cannot determine individual compounds.

- Sensitivity for dioxins in sub-ppb range in water and soil, low ppb to ppm range for PCBs and PAHs.
NEW DIOXIN AND COPLANAR PCB METHODS

- Enzyme Immunoassays (EIA) from Cape Technologies.
- Results based on Toxicity Equivalence Factors (TEF) or Toxicity Equivalence (TEQ) with respect to 2,3,7,8-TCDD.
- Dioxin method (Method 4025) and Coplanar PCB method (Method 4026) are in final stages of validation and field testing.
COPLANAR PCB METHOD

• Method 4026

  – Sensitive to the 14 PCB congeners which have TEFs assigned because of their coplanar dioxin-like structures.

  – Uses PCB 126 (3,3',4,4',5-pentachlorobiphenyl) as the primary target analyte.
COPLANAR PCB METHOD
continued

– Not sensitive to the common PCB congeners in Aroclor 1254.

– Sensitivity down to 15 pg TEQ per µg Aroclor.
DIOXIN METHOD

• Method 4025

• Sensitivity based on TEQ of 2,3,7,8-TCDD.

• Used for screening soil samples at 500 ppt. Water method currently under evaluation.
DIOXIN METHOD
continued

• 91% correct identifications, 9% false positive rate, 0% false negative rate in field study on 56 real world samples previously characterized using GC/HRMS.

• Significant cost saving potential for analyses involving dioxin cleanups.
MERCURY ANALYSIS BY IMMUNOASSAY

- Method 4500 - developed by BioNebraska
- ELISA technique
- Action level to 0.5 ppm Hg
- Acid extraction with HCl, HNO₃, and water
- Colorimetric determination by IA
Delfia Method for Dioxins

- Method 4430 developed by Hybrizyme.
- Dissociation-enhancement lanthanide fluor-immunoassay.
- Utilizes Ah receptor response for dioxin TEQ.
- Non-competitive immunoassay.
- Very good sensitivity for target analytes.
NEW DEVELOPMENT PROJECTS

• Continued development of 2nd generation
  – Pesticides and other compound classes.
  – Development of more quantitative methods from the very selective screening products, e.g., 2,4-D, Silvex, explosives.
  – New kit formats, similar to home pregnancy test kits.
  – New kits for metals, e.g., Pb, Cd
OTHER NEW DEVELOPMENT PROJECTS

• Working with Hybrizyme to complete development of their TEQ dioxin method using the AH receptor.

• Use of selectivity of immunoassays in sample preparative mode through affinity chromatography.