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

Method Analyte	Manufacturer						
	A	B	C	D	E	F	G
4010 PCP				Water Soil	Water Soil	Soil	
4015 2,3-D					Water Soil	Water Soil	
4020 PCB			Soil	Soil Oil	Soil	Soil	Soil
4025 Dioxins		Soil					
4030 TPH				Soil	Soil	Soil	
4035 PAH			Soil	Soil	Soil	Soil	

Approved Immunoassay Methods

Method Analyte	Manufacturer						
	A	B	C	D	E	F	G
4040 Toxaphene					Soil		
4042 DDT					Soil		
4050 TNT					Soil	Soil	Soil
4051 RDX							Soil
4500 Mercury	Soil						
4670 Triazines						Water	

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, HNO_3, and water
- Colorimetric determination by IA

DELFIA METHOD FOR DIOXINS

- Method 4430 developed by Hybrizyme.
- Dissociation-enhancement lanthanide fluoroimmunoassay.
- 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.