PRINCIPLES AND PROSPECTS FOR BIOREMEDIATION OF PCBs IN SOILS AND SEDIMENTS

James M. TIEDJE
Distinguished Professor and Director
Center For Microbial Ecology
Michigan State University
tiedjej@msu.edu

1

Current Biological Principles For Bioremediation Of PCBs

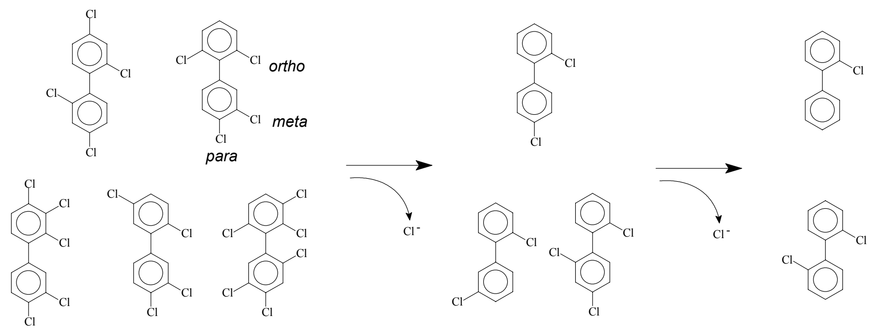
- Highly chlorinated PCBs (Aroclors) can be reduced to a lesser chlorinated species by anaerobic dehalogenating communities
- The lesser chlorinated PCB species can be oxidized by aerobic biphenyl degraders yielding chlorinated benzoates, pentadienes and HOPDAs
- Chlorobenzoates can be mineralized by yet another group of bacteria
- These three independently occurring processes can be combined in a singular two-phase anaerobic-aerobic remediation scheme

2

Rationale for anaerobic/aerobic biotreatment

Less aerobically degradable

More aerobically degradable



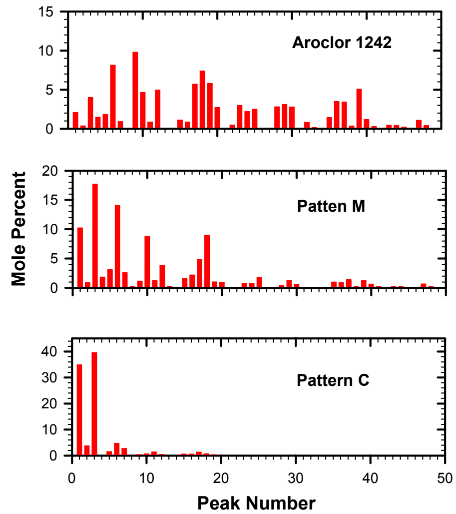
Some components
of Aroclor 1242
(commercial PCB mixture)

Intermediate
dechlorination products

Advanced
dechlorination products

3

Chromatographic Profiles - Pattern



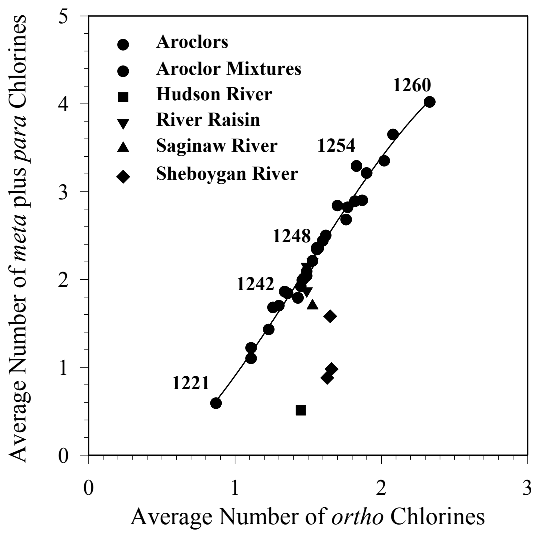
4

Congener Specificity

Dechlorination Activity	Susceptible Chlorines
M	Flanked & unflanked *meta*
Q	Flanked & unflanked *para*
H	Flanked *para* Doubly flanked *meta*
H’	Flanked *para* *Meta* of 2,3- & 2,3,4- groups
P	Flanked *para*
N	Flanked *meta*
T	*Meta* of hepta- & octa-CBs
LP	Unflanked *para*

5

Evaluation of *in situ* PCB Dechlorination



6

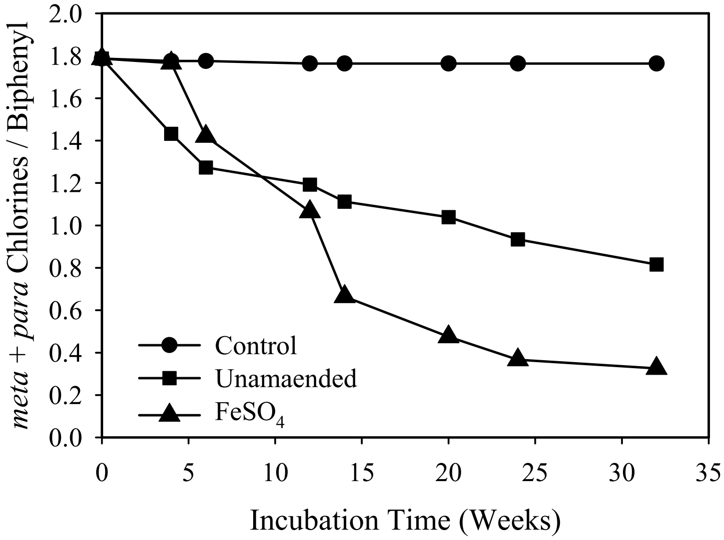
Occurrence of PCB Dechlorination

- Acushnet Estuary (MA)
- Hudson River
- Industrial lagoons
- River Raisin (MI)
- Sheboygan River (WI)
- Silver Lake (MA)
- Escambia Bay (FL)
- Hoosic River (MA)
- Lake Ketelmeer/Rhine River (Netherlands)
- Waukegan Harbor (IL)
- Wood's Pond (MA)

Observed extent of dechlorination tends to taper off below 50 to 100 ppm.

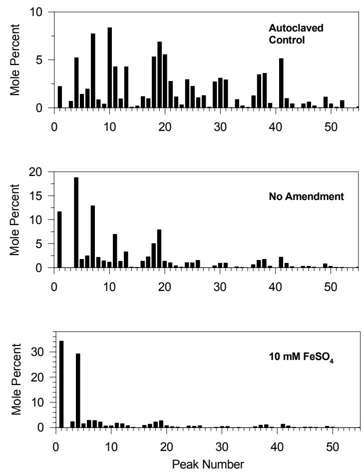
7

$FeSO_4$ Amendment
Extent of Dechlorination



8

Effect of $FeSO_4$ on Aroclor 1242 Dechlorination by Hudson River Microorganisms



No amendment:

- Process M (*meta*) dechlorination
- 25% of Cl removed
- *Ortho* & *para* substituted products:
  - 2-CB, 2,2’-CB, 26-CB, 2,4’-CB, 2,2’,4-CB, 2,4,4’-CB

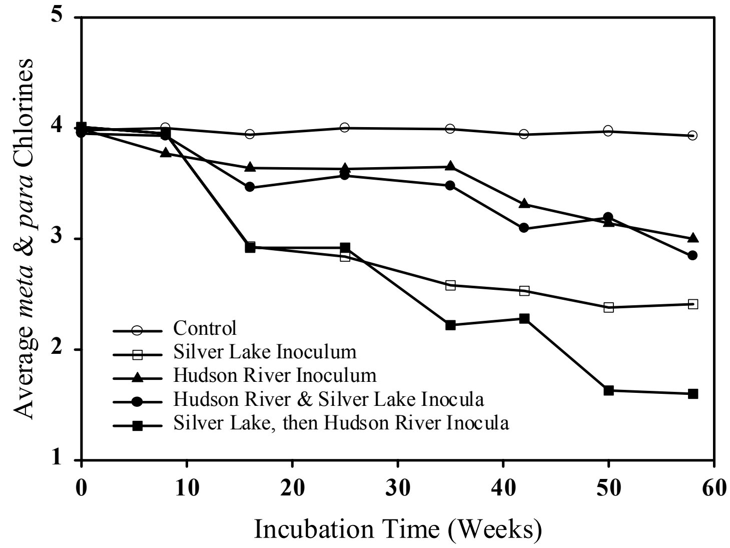
With $FeSO_4$:

- Adds process Q (*para)* dechlorination
- 50% of Cl removed
- *Ortho* substituted products:
  - 2-CB, 2,2’-CB, 2,6-CB

9

Sequential Inoculations

Extent of Dechlorination



10

Importance of PCB Dechlorination

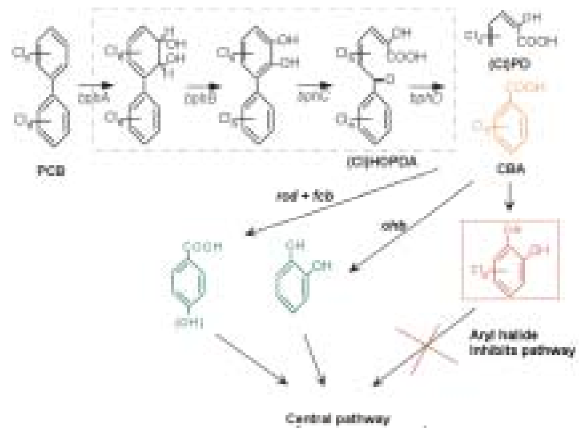
- Is an intrinsic process
- Has potential for bioremediation
- Products are more aerobically degradable
- Products are generally less toxic
- But, degradation is usually incomplete with especially *ortho*-PCBs remaining, hence, an aerobic phase is needed

11

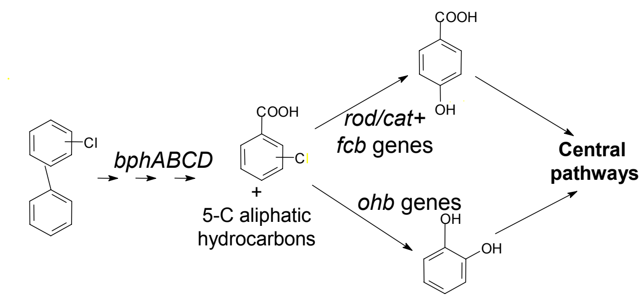
Barriers to Aerobic Biodegradation

- Limited to lesser chlorinated congeners
- Co-metabolic
  - Requires induction
  - Yields no growth
- Incomplete - accumulates potentially problematic compounds

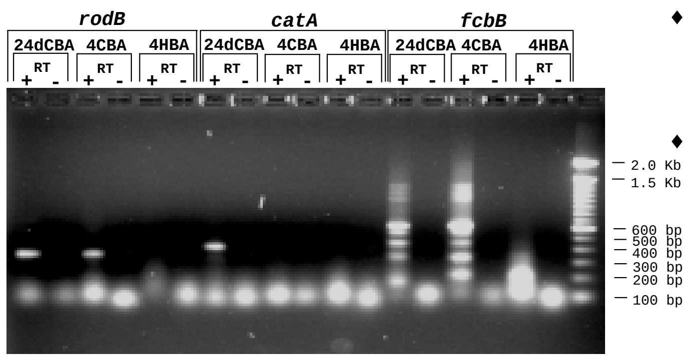
Recombinant PCB degradation Pathways



13



- **Halohydroxyperoxidase/ catalase (*catA*) to use for upgrading pathway for *ortho* + *para*-PCBs**
- **The *catA* is related to (bromo) hydroxyperoxidases for anoxic chlorination/dechlorination of chloramphenicol**
- **The laboratory evolution to target enzyme(s) for reductive dechlorination of both CBAs and PCBs**
- **The *catA* family members to use for the combinatorial mutagenesis**



Differential expression of dehalogenases in response to CBAs.
Reverse transcription (RT)-PCR of total RNA from Nocardioides sp. KZ4N.

14

Anaerobic Reductive Dechlorination

$FeSO_4$ →
- Rates/Conc.

Surfactants →
- Sorption/Availability

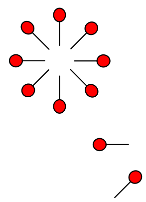
← **Seed Inoculum**
- Mixed culture

↓

Aerobic PCB Dechlorination And Growth

Genes →
ohb, fcb, rod, clc
G+/- Cassette

Surfactants →
Toxicity/Growth



← **Organisms**
Burkholderia sp. LB400(ohb) (G-)
-Rhodococcus erythropolis RHA1(fcb) (G+)

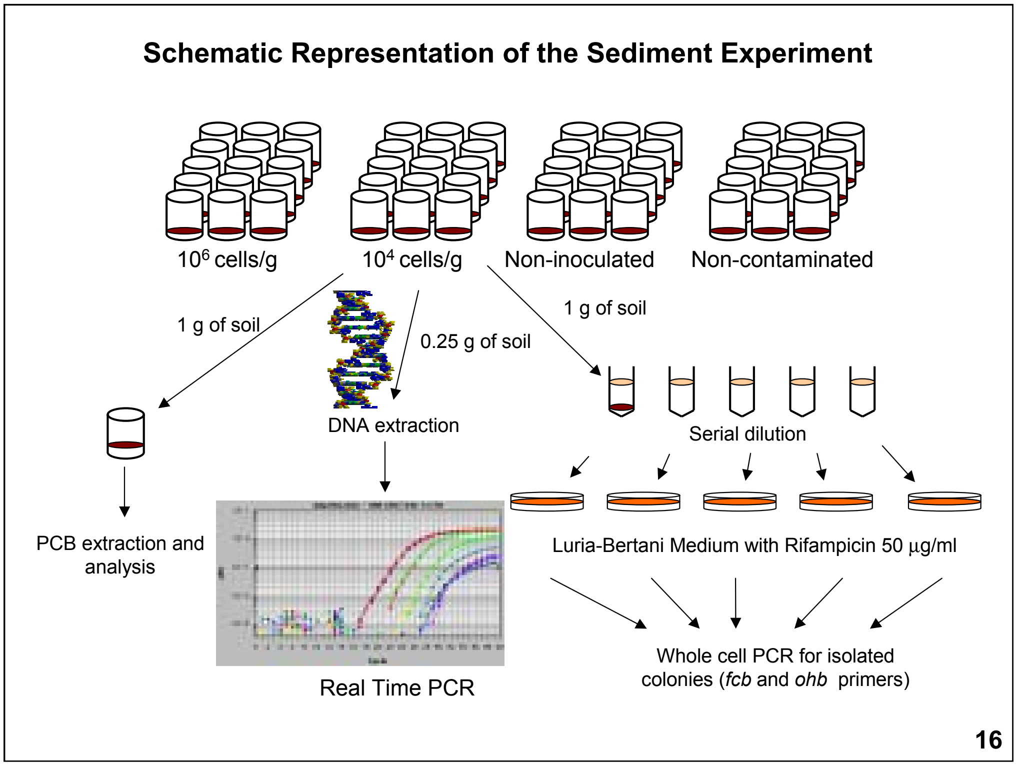
← **Inocula Technology**
Carrier Media

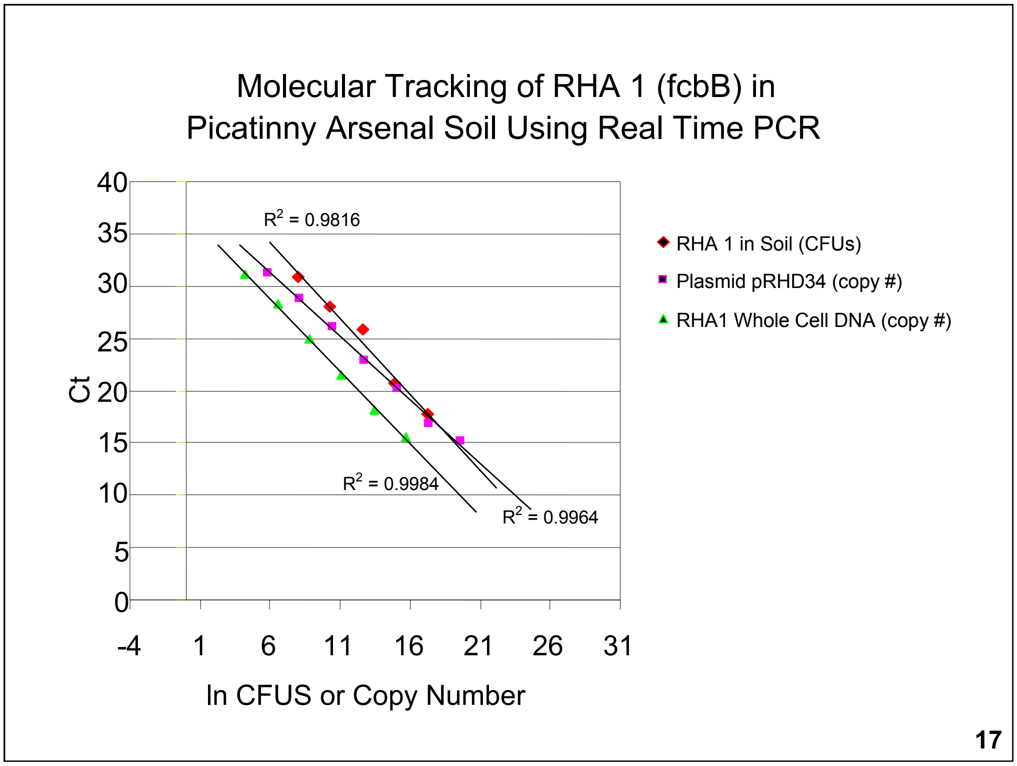
↓

PCB Remediation Toolbox

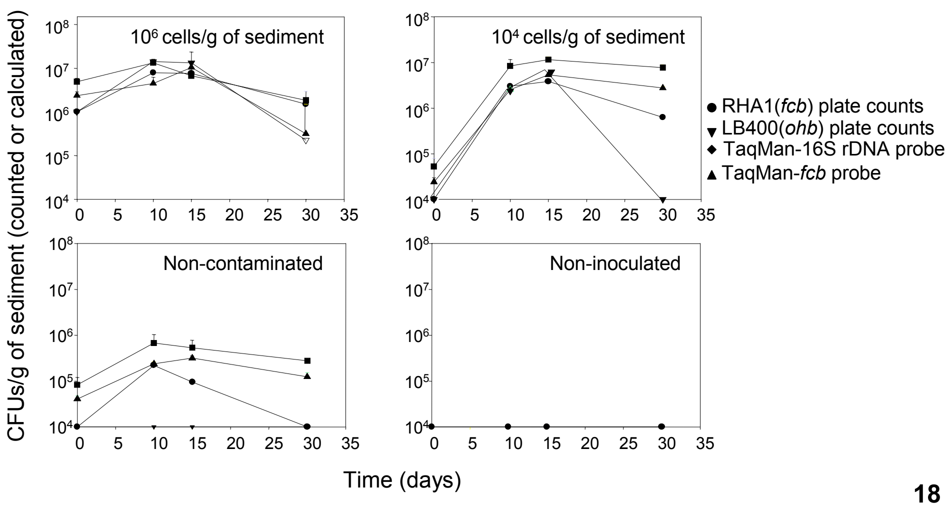
- **Ex-Situ BioReactor**
  -low, medium, high
  -density solids

15

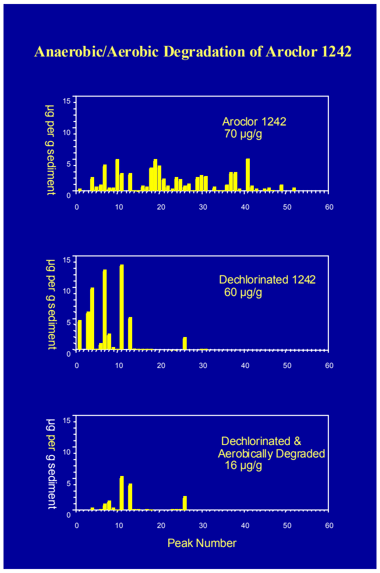

Schematic Representation of the Sediment Experiment
10^6 cells/g
10^4 cells/g
Non-inoculated
Non-contaminated
1 g of soil
0.25 g of soil
1 g of soil
DNA extraction
Serial dilution
PCB extraction and analysis
Luria-Bertani Medium with Rifampicin 50 µg/ml
Whole cell PCR for isolated colonies (fcb and ohb primers)
Real Time PCR
16


Molecular Tracking of RHA 1 (fcbB) in
Picatinny Arsenal Soil Using Real Time PCR
R^2 = 0.9816
R^2 = 0.9984
R^2 = 0.9964
Ct
ln CFUS or Copy Number
RHA 1 in Soil (CFUs)
Plasmid pRHD34 (copy #)
RHA1 Whole Cell DNA (copy #)
40
35
30
25
20
15
10
5
0
-4
1
6
11
16
21
26
31
17

Population dynamics of RHA1(*fcb*) and LB400(*ohb*) during aerobic treatment of Aroclor 1242 anaerobic dechlorination products (Red Cedar River sediment)



18



Laboratory scale two-phase PCB remediation (Red Cedar River sediment)

- **Enhanced anaerobic dechlorination of Aroclor resulted in shifting from highly to lower chlorinated congeners**
- **Bioaugmentation with engineered aerobic G+/G- GEM cassette LB400(*ohb*)+RHA1(*fcb*) resulted in efficient degradation of the remaining PCBs**

19

PCB-growing Dechlorinating GEM Advantages

- Increased biomass
  smaller inoculum
- Reduced HOPDA
  diminished toxicity
- Issues remaining: Still incomplete mineralization of higher chlorinated PCB species
  lower degradation rates, incomplete Cl-HOPDA removal

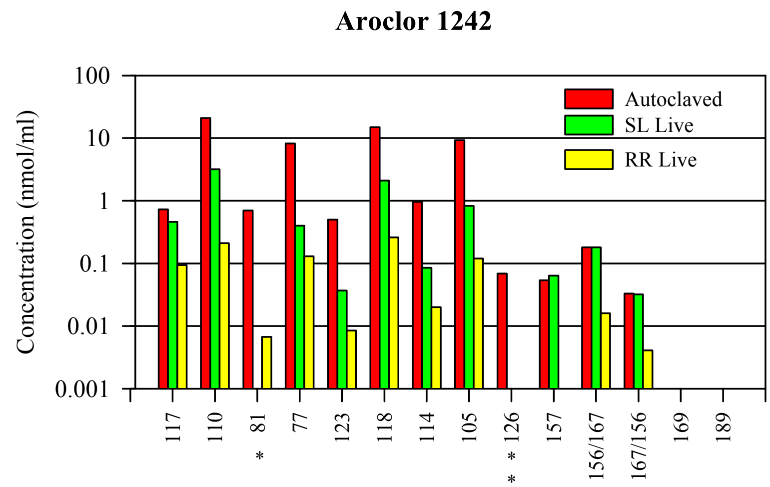
Effect of the *ohb* (pRO41) and *fcb*(pRHD34) genes on PCB degradation

Strain(plasmid)/ inducer	Mix M, nom. 1mM				Mix C, nom. 1 mM			
	% PCB degraded	OD_{600}	Cl^- Release, mM	HOPDA λ=394	% PCB degraded	OD_{600}	Cl^- Release, mM	HOPDA λ =394
$LB400(pRT1)_{Bph}$	83.8	**0.19**	1.33	**0.476**	93.7	**0.21**	0.91	**0.066**
$LB400(pRO41)_{25CBA}$	75	**0.24**	1.98	**0.164**	93.2	**0.35**	1.47	**Not detected**
$LB400(pRO41)_{25CBA}$ + $RHA1(pRHD34)_{4CBA}$	67.6	0.19	1.5	**0.139**	91.3	**0.30**	1.42	**0.069**

20

Biological Activity

Dechlorination of Congeners with AhR-Mediated Activity



21

AhR Mediated Toxicity Reduction

Sample	TEQ_{assay}*	TEQ_{calc}*
Non-dechlorinated 1242	3.1	5.7
SL dechlorinated 1242	<6 X 10-2**	7.8 X 10-2
RR dechlorinated 1242	<6 X 10-2**	4.7 X 10-2

*Units are pmole TEQ/∑ µmoles of PCBs.
**TEQ_{assay} was less than the MDL.

22

Biological Activity Summary

Biological Activity	Anaerobic Dechlorination	Aerobic Di-OHs
AhR mediated	Reduced/eliminated	Inactive
AP-transcription	Reduced/eliminated	Inactive
Fertilization	Reduced/eliminated	----------
Neutrophil activation	Unaffected	Inactive
Insulin release	Reduced/eliminated	Decreased
Estrogenicity	-----------	Inactive
Uterine contractility	Increased	----------

23

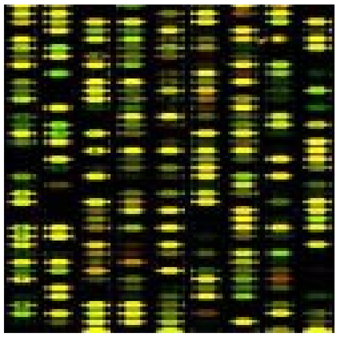
Genome of *Burkholderia* Strain LB400

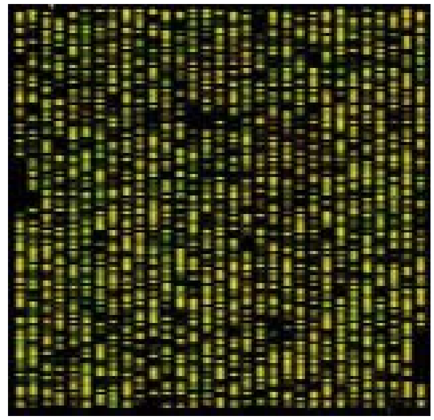
- Best known PCB degrader sequenced
- 9.5 Megabases, largest bacterial genome yet sequenced
- Has multiple chromosomes
- Has many aromatic degradation genes

24

Differential Expression by a PCB Degrader

4,000 genes of
Burkholderia strain LB400





Genes (mRNA) expressed during growth on succinate (green, Cy3) vs. genes expressed during growth or rich media (red, Cy5), and expressed under both conditions (yellow)

25

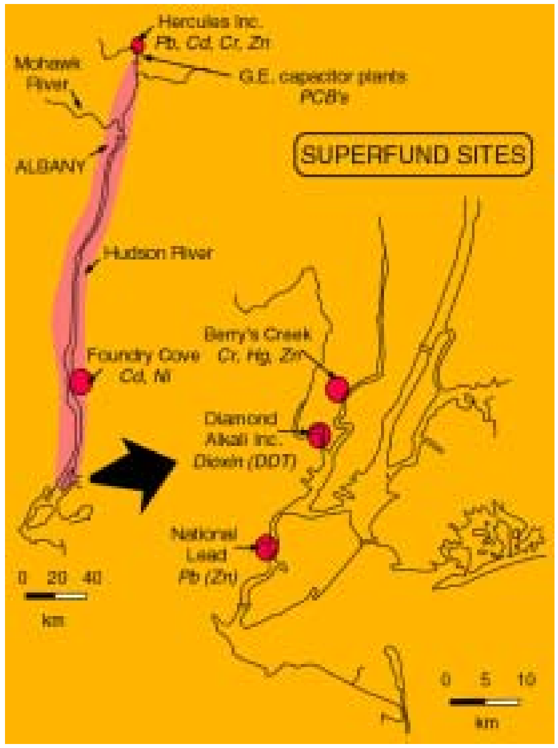
Hudson River PCBs: Lessons from Dated Sediment Cores

Richard Bopp and Edward Shuster, Rensselaer Polytechnic Institute
Steven Chillrud, Lamont Doherty Earth Observatory of Columbia University
Frank Estabrooks, New York State Department of Environmental Conservation

NIEHS Superfund Basic Research Program
Grant to Mount Sinai Medical Center
Philip J. Landrigan, P.I.

OCTOBER 2002

26


Hercules Inc.
Pb, Cd, Cr, Zn
Mohawk River
G.E. capacitor plants
PCB's
ALBANY
SUPERFUND SITES
Hudson River
Berry's Creek
Cr, Hg, Zn
Foundry Cove
Cd, Ni
Diamond
Alkali Inc.
Dioxin (DDT)
National
Lead
Pb (Zn)
0 20 40
km
0 5 10
km

27

28

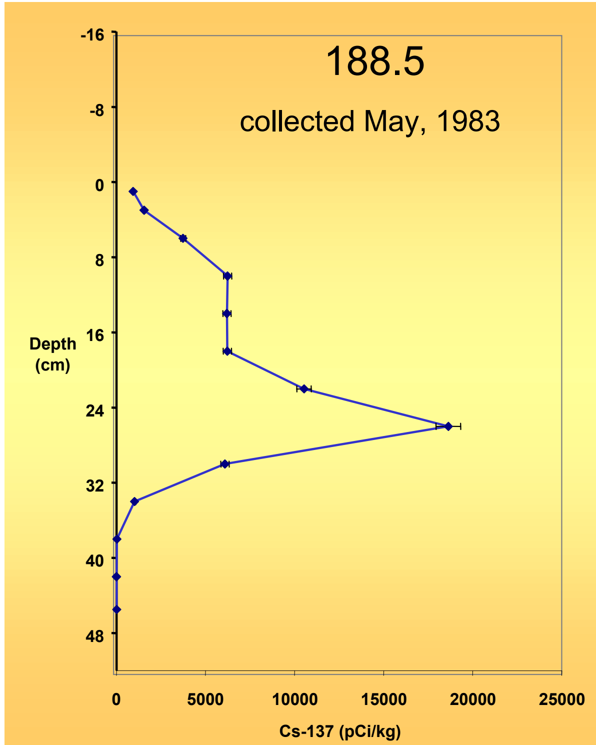
29

Basics of Our Radionuclide Dating of Sediment Cores

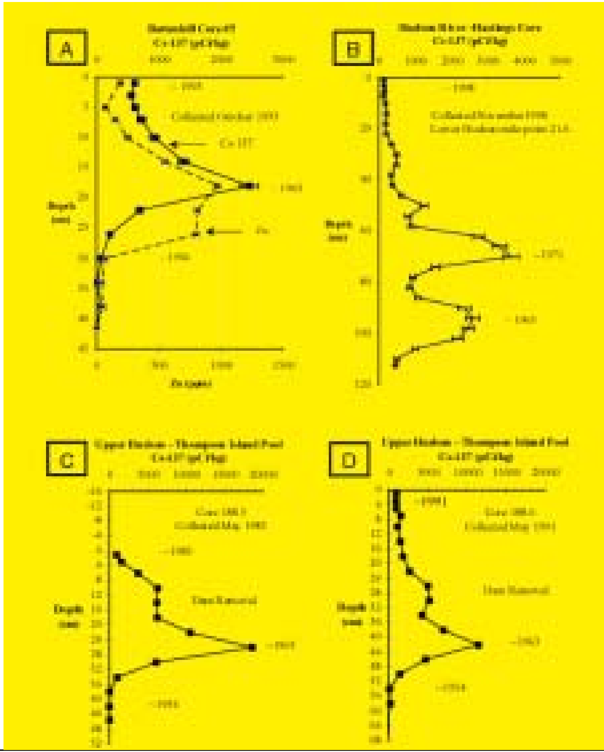
•Cs-137 - supplied by global fallout from atmospheric testing of nuclear weapons. In "ideal" sediment cores the Cs-137 profile provides at least two time horizons - a "first appearance" in about 1954 and a maximum activity in 1963-64.

•Be-7 - a short-lived natural radionuclide (half life = 53 days). Detection of Be-7 in near surface sections of sediment cores confirms the presence of a significant component of particles deposited within about a year of core collection.

30

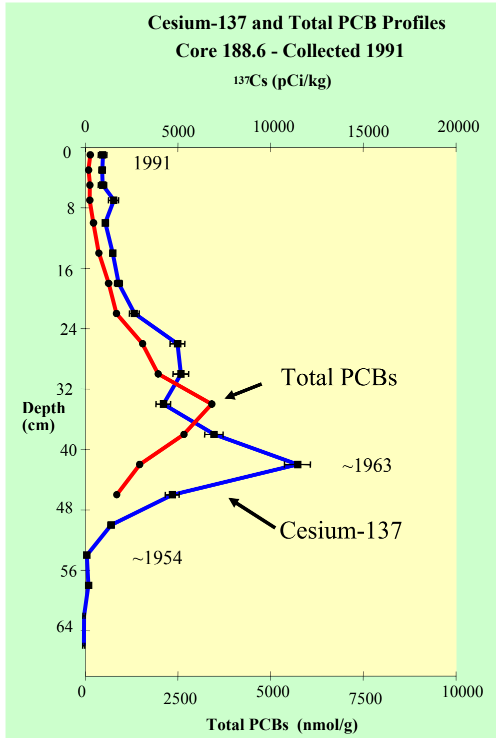

188.5
collected May, 1983
Depth (cm)
-16
-8
0
8
16
24
32
40
48
0
5000
10000
15000
20000
25000
Cs-137 (pCi/kg)

31



"Well-dated" cores have been collected from the Hudson at mile points 203, 193.8, 188.5, 177.6, 163.6, 152.7, 91.8, 88.6, 59, 54, 43, 21.6, 3.0, and -1.7. Samples of all core sections have been archived.

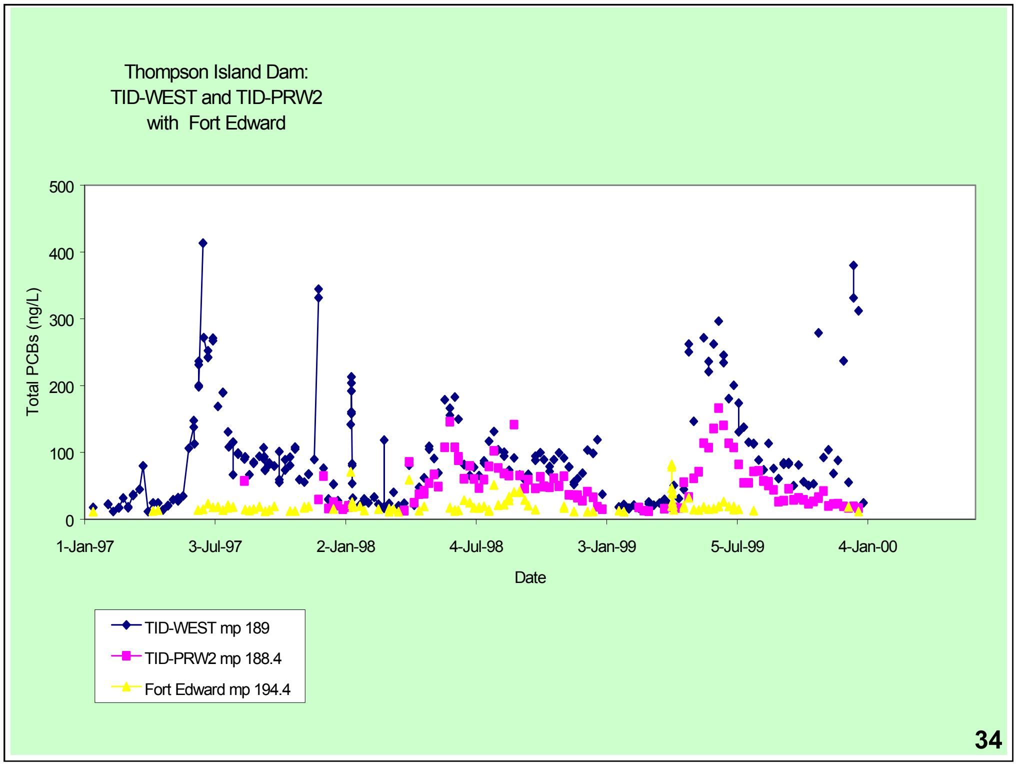
32



Despite major "dilution" with cleaner particles from tributaries, the early 1970s peak in PCB levels can be traced downstream to the NY/NJ Harbor.

The best evidence for continuing transport of PCBs from sediments of the Thompson Island Pool was provided by weekly water column monitoring.

33

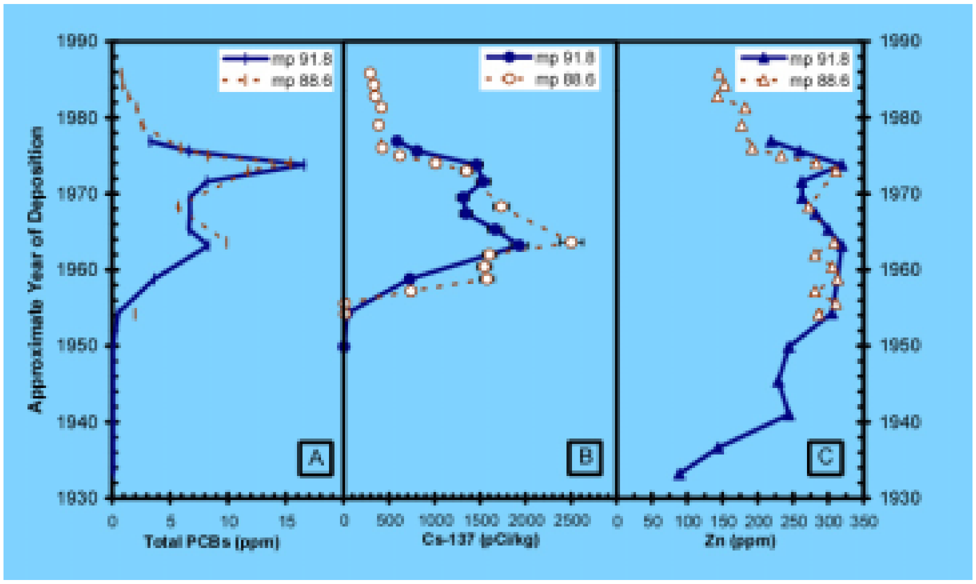

Thompson Island Dam:
TID-WEST and TID-PRW2
with Fort Edward
Total PCBs (ng/L)
500
400
300
200
100
0
1-Jan-97
3-Jul-97
2-Jan-98
4-Jul-98
3-Jan-99
5-Jul-99
4-Jan-00
Date
TID-WEST mp 189
TID-PRW2 mp 188.4
Fort Edward mp 194.4
34

In situ reductive dechlorination of PCBs has been studied using paired samples from co-located sediment cores.

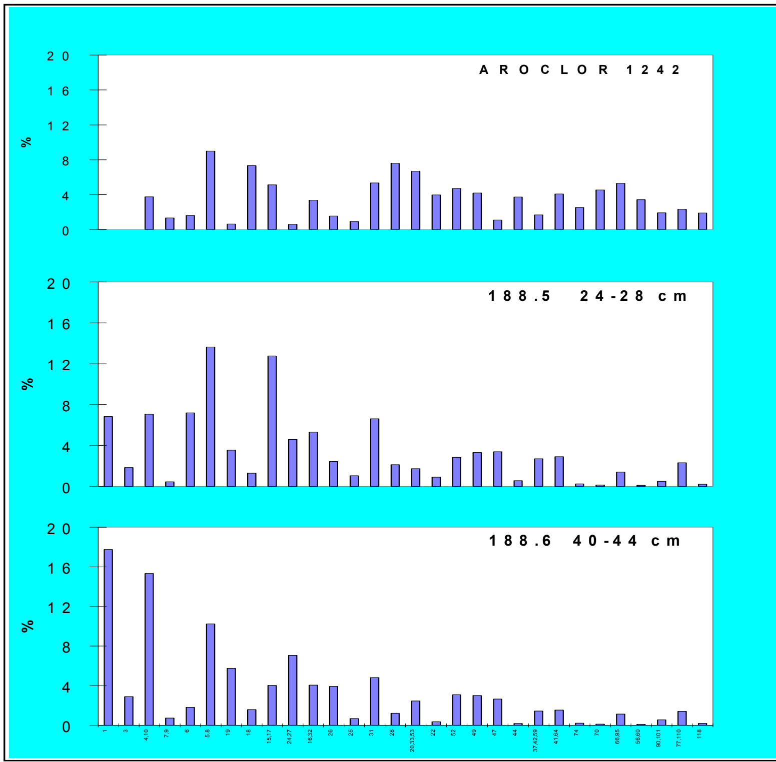
•This process results in dramatic alteration of PCB composition in upper Hudson sediments, but has not progressed as far as lab studies would suggest.

•In sediments from the freshwater reach of the tidal Hudson, reductive dechlorination can be easily detected, but the overall effect on PCB composition has been minor.

Matching contaminant profiles in paired, co-located cores



36



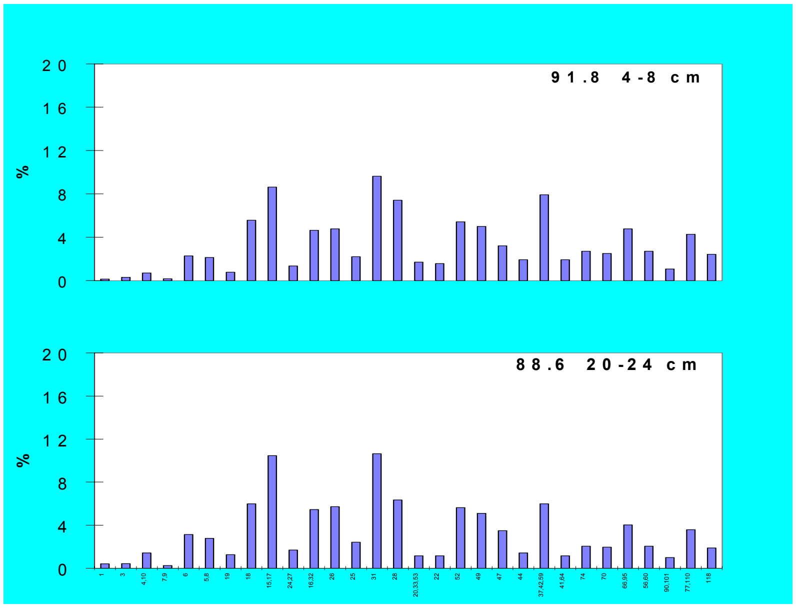
Aroclor 1242

Thompson Island Pool -

deposited 1963
sampled 1983

deposited 1963
sampled 1991

37

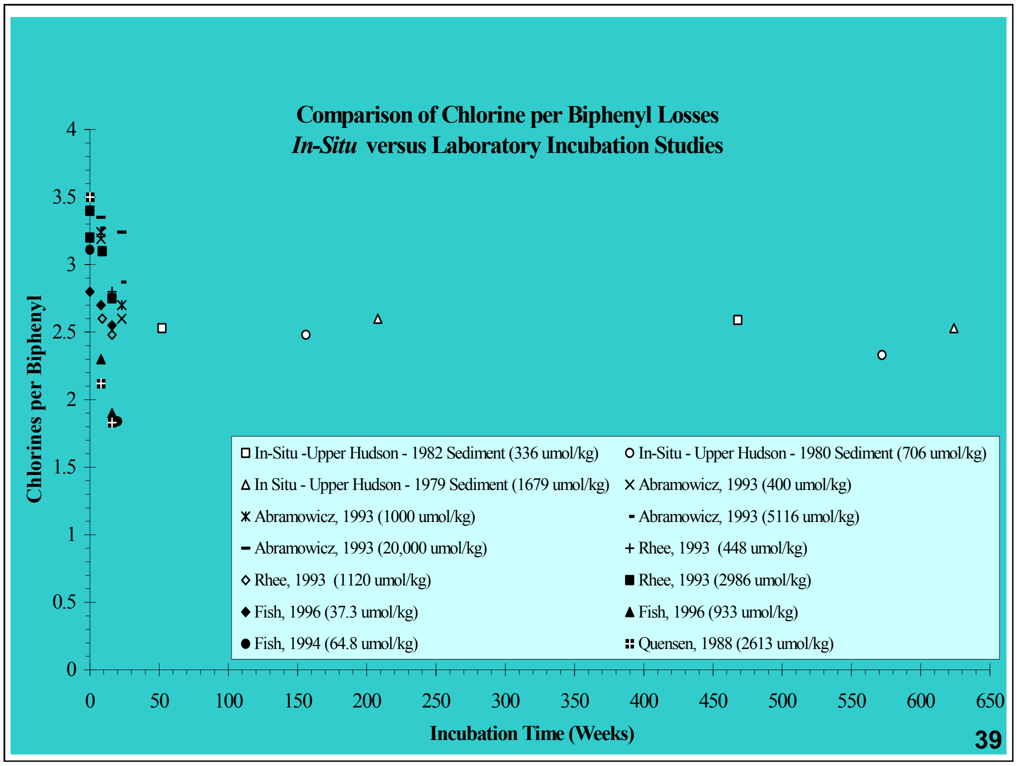


Tidal Hudson -

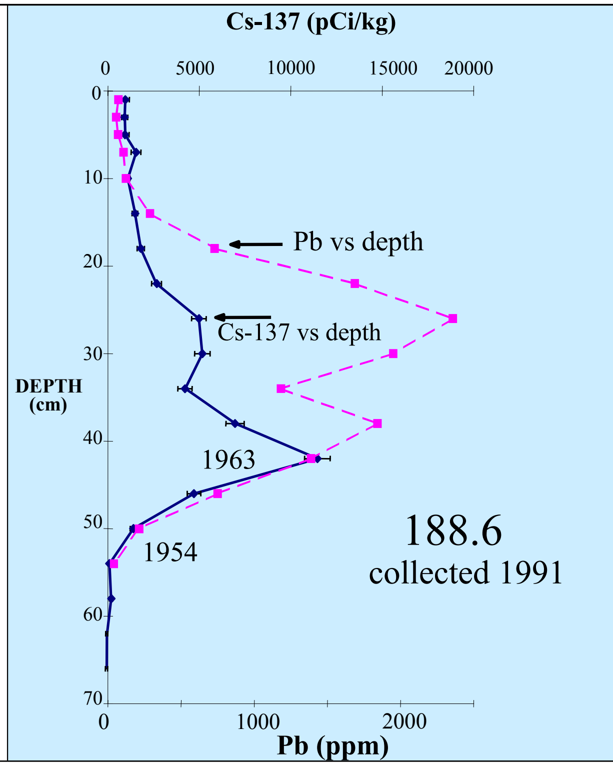
deposited 1973
sampled 1977

deposited 1973
sampled 1986

38

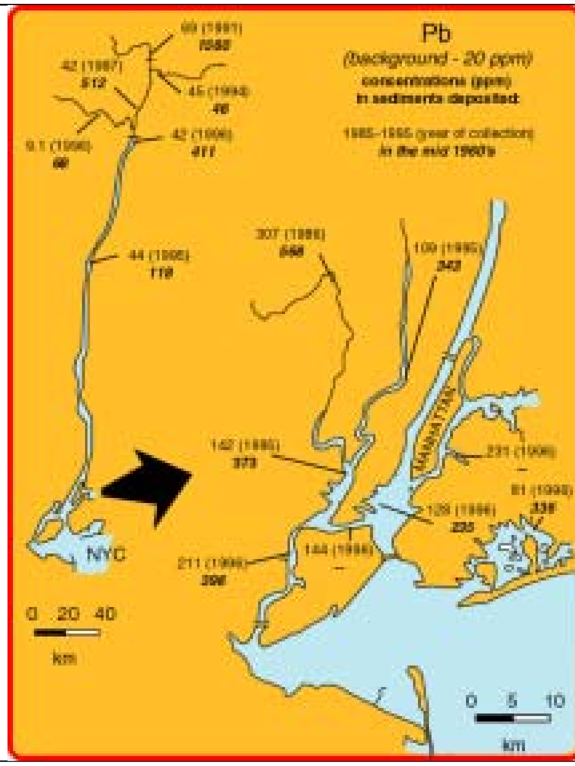

Comparison of Chlorine per Biphenyl Losses
In-Situ versus Laboratory Incubation Studies
Chlorines per Biphenyl
Incubation Time (Weeks)
In-Situ -Upper Hudson - 1982 Sediment (336 umol/kg)
In-Situ - Upper Hudson - 1980 Sediment (706 umol/kg)
In Situ - Upper Hudson - 1979 Sediment (1679 umol/kg)
Abramowicz, 1993 (400 umol/kg)
Abramowicz, 1993 (1000 umol/kg)
Abramowicz, 1993 (5116 umol/kg)
Abramowicz, 1993 (20,000 umol/kg)
Rhee, 1993 (448 umol/kg)
Rhee, 1993 (1120 umol/kg)
Rhee, 1993 (2986 umol/kg)
Fish, 1996 (37.3 umol/kg)
Fish, 1996 (933 umol/kg)
Fish, 1994 (64.8 umol/kg)
Quensen, 1988 (2613 umol/kg)
39

“Other” significant contaminants in upper Hudson sediments include Pb, Cd, Cr, Zn, and dioxins.

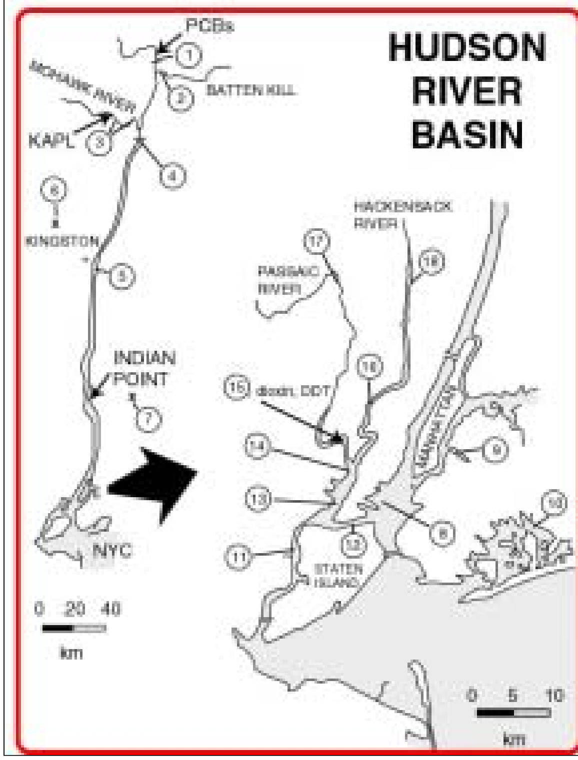


40

A basin-wide perspective on Pb



41



The importance of other PCB sources can be determined from analyses of dated sediments from "indicator" sites. Site 9 is Newtown Creek, a municipal wastewater indicator. Sites 6 & 7 (NYC drinking water reservoirs) and Central Park Lake are atmospheric deposition indicators.

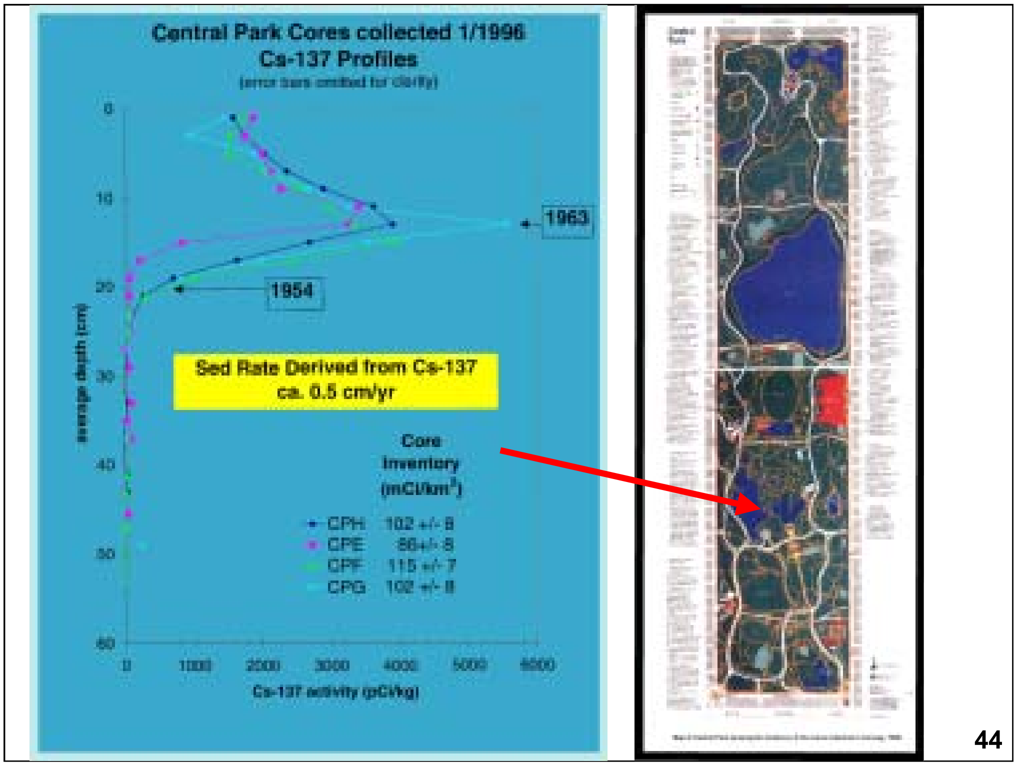
42

Table 1. Contaminant levels in sediment samples deposited in the mid 1980s.

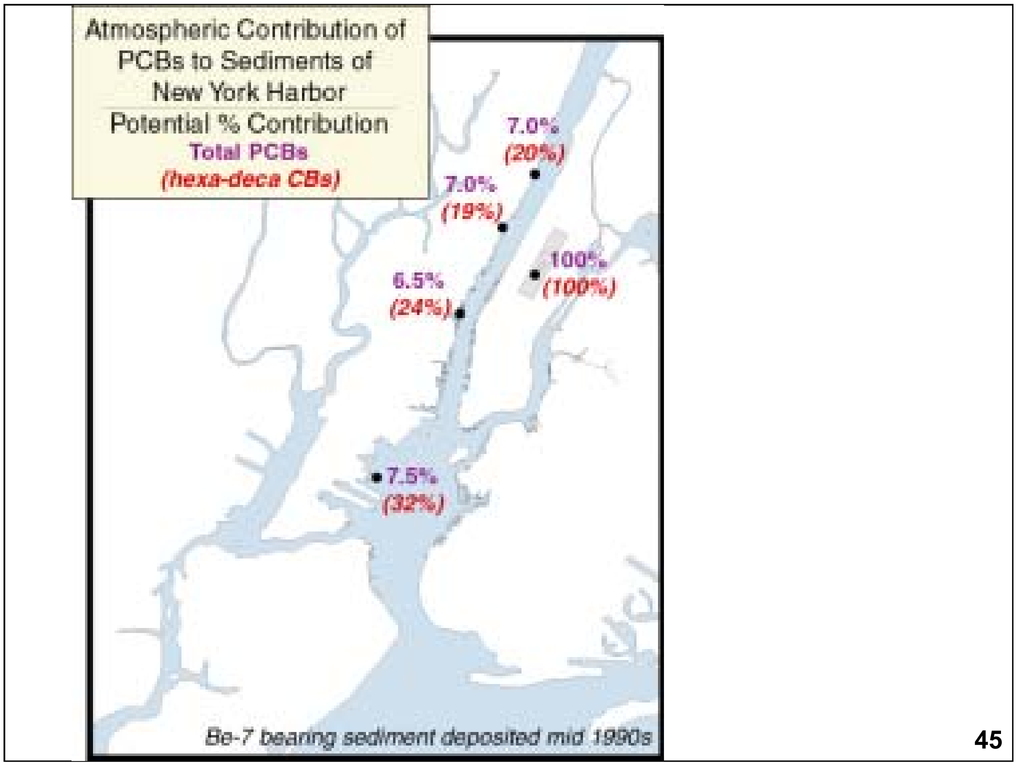
	Newtown Creek site 9	NY/NJ harbor site 8	Kingston site 5
Organic matter (%)[a]	12.8	9.5 (64%)[b]	3.6
pp'-DDD (ppb)	62	35 (52%)	6.2
γ-chlordane (ppb)	28	16 (57%)	<1
total PCBS (ppm)	1.91	1.47 (61%)	0.79
copper (ppm)	604	207 (31%)	31
lead (ppm)	466	223 (41%)	53
zinc (ppm)	748	358 (35%)	145
cadmium (ppm)	10.5	4.03 (31%)	1.12

[a]Based on weight loss on ignition at 375°C overnight.

[b]Values in parentheses represent the sewage contribution to contaminant levels in this sample calculated as ((NY/NJ harbor – Kingston)/ (Newtown Creek – Kingston)) x 100.



Central Park Cores collected 1/1996
Cs-137 Profiles
(error bars omitted for clarity)
average depth (cm)
0
10
20
30
40
50
60
0
1000
2000
3000
4000
5000
6000
Cs-137 activity (pCi/kg)
1963
1954
Sed Rate Derived from Cs-137
ca. 0.5 cm/yr
Core
Inventory
(mCi/km^2)
CPH 102 +/- 8
CPE 66+/- 8
CPF 115 +/- 7
CPG 102 +/- 8
44


Atmospheric Contribution of PCBs to Sediments of New York Harbor
Potential % Contribution
Total PCBs
(hexa-deca CBs)
7.0%
(20%)
7.0%
(19%)
100%
(100%)
6.5%
(24%)
7.5%
(32%)
Be-7 bearing sediment deposited mid 1990s
45

Conclusions:

•Analyses of sections from dated sediment cores is the single most efficient means of understanding the sources, distribution, and fate of particle-associated contaminants in natural water systems. (So I'm biased.)

•A multi-contaminant/tracer approach provides critical support for any interpretation of the behavior of any specific particle-associated contaminant (PCBs) in a natural water system (the Hudson).

Recent significant developments in the Hudson River PCB story include stable lead isotopes as tracers of upper Hudson particles; plutonium isotopes as tracers of Mohawk River particles; and stable isotopes as tracers of reductive dechlorination of PCBs.

46

Thank You

[Links to Additional Resources]