Stimulating Microbial Dechlorination of PCBs *in situ*

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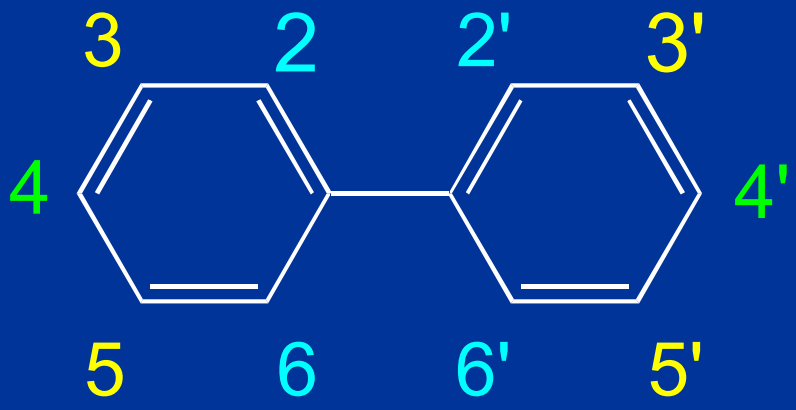
Polychlorinated Biphenyls (PCBs)



ortho *2, 6*

meta *3, 5*

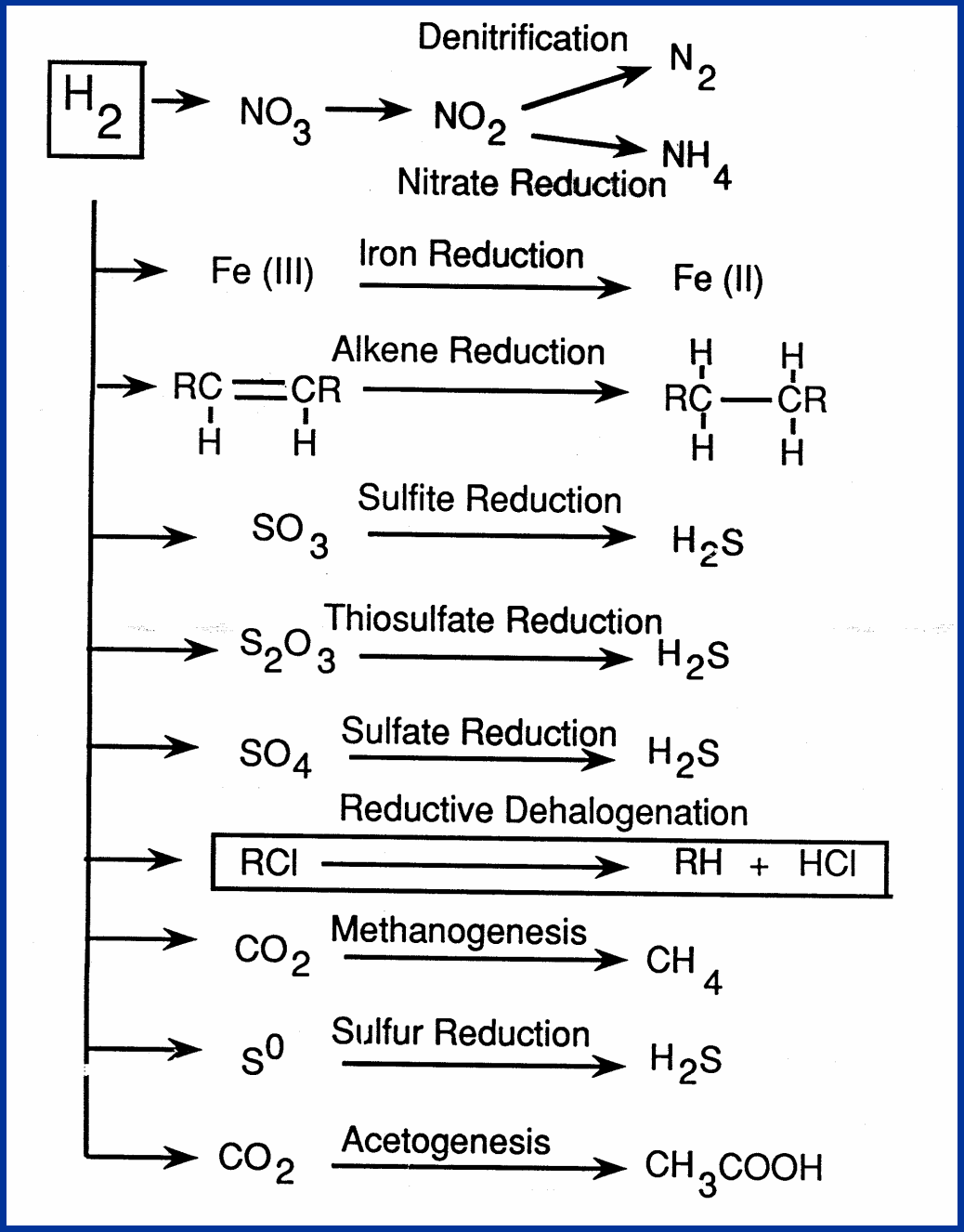
para *4*

U.S. Sites Containing PCB-Dechlorinating Microorganisms

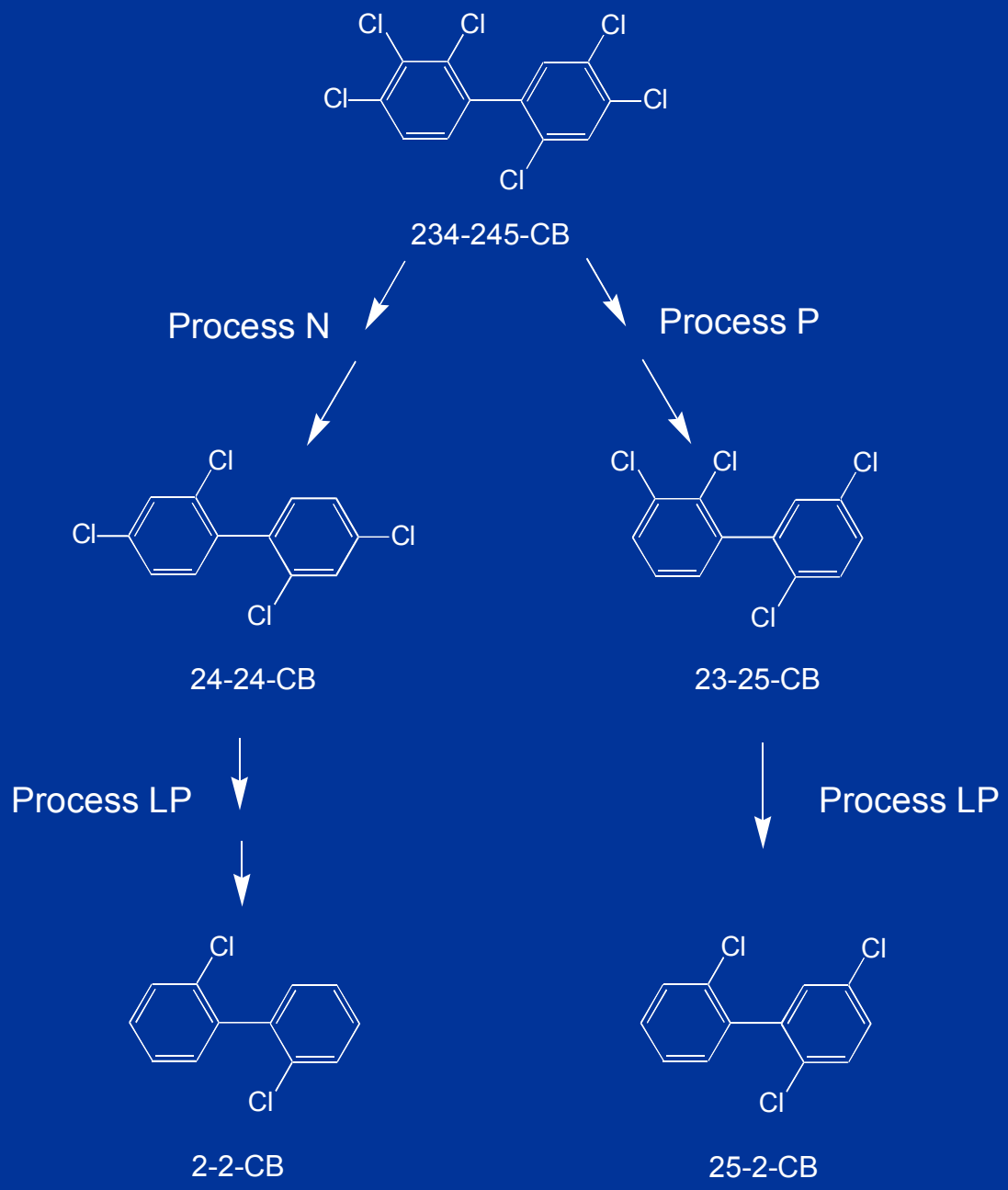
LOCATION	PCB-CONTAMINATED SITE
Florida	Escambia Bay
Georgia	LCP Superfund Site (Brunswick)
Illinois	Waukegan Harbor
Maryland	Baltimore Harbor
Massachusetts	Hoosic River, Silver Lake, New Bedford Harbor
	Housatonic River / Woods Pond
Michigan	River Raisin
South Carolina	Charleston Harbor, Lake Hartwell
Washington	Puget Sound
Wisconsin	Fox River, Sheboygan Harbor and River

What Influences Microbial PCB Dechlorination?

- PCB concentration
- PCB congener distribution
  - Penta- and Hexa-CBs, multiple flanked chlorines
  - Tri- and Tetra-CBs, few flanked chlorines
  - Hepta- Nona-CBs , less soluble, steric hindrance
- Environmental Conditions: Temperature, pH
- Electron donors: H_2, organic compounds
- Electron acceptors: sulfate, iron, nitrate, carbonate
- Co-contaminants:
  - Oil, organics
  - Pesticides, xenobiotics
  - Heavy metals



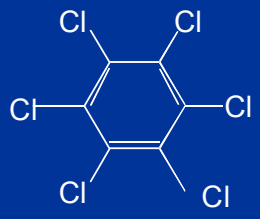
Electron Accepting Reactions in Anaerobic Environments



Three Different Dechlorination Processes in Woods Pond:

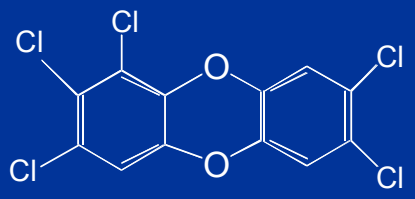
N, P, and LP

Halorespiration

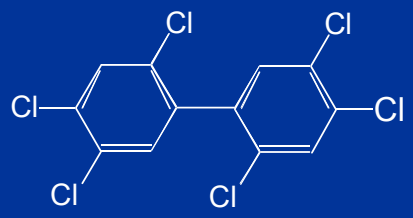


CHLOROBENZENE

CHLOROPHENOL



1,2,3,7,8-PeCDD



PCBs ?

- Some bacteria require halogenated aromatics for growth

Priming PCB Dechlorination

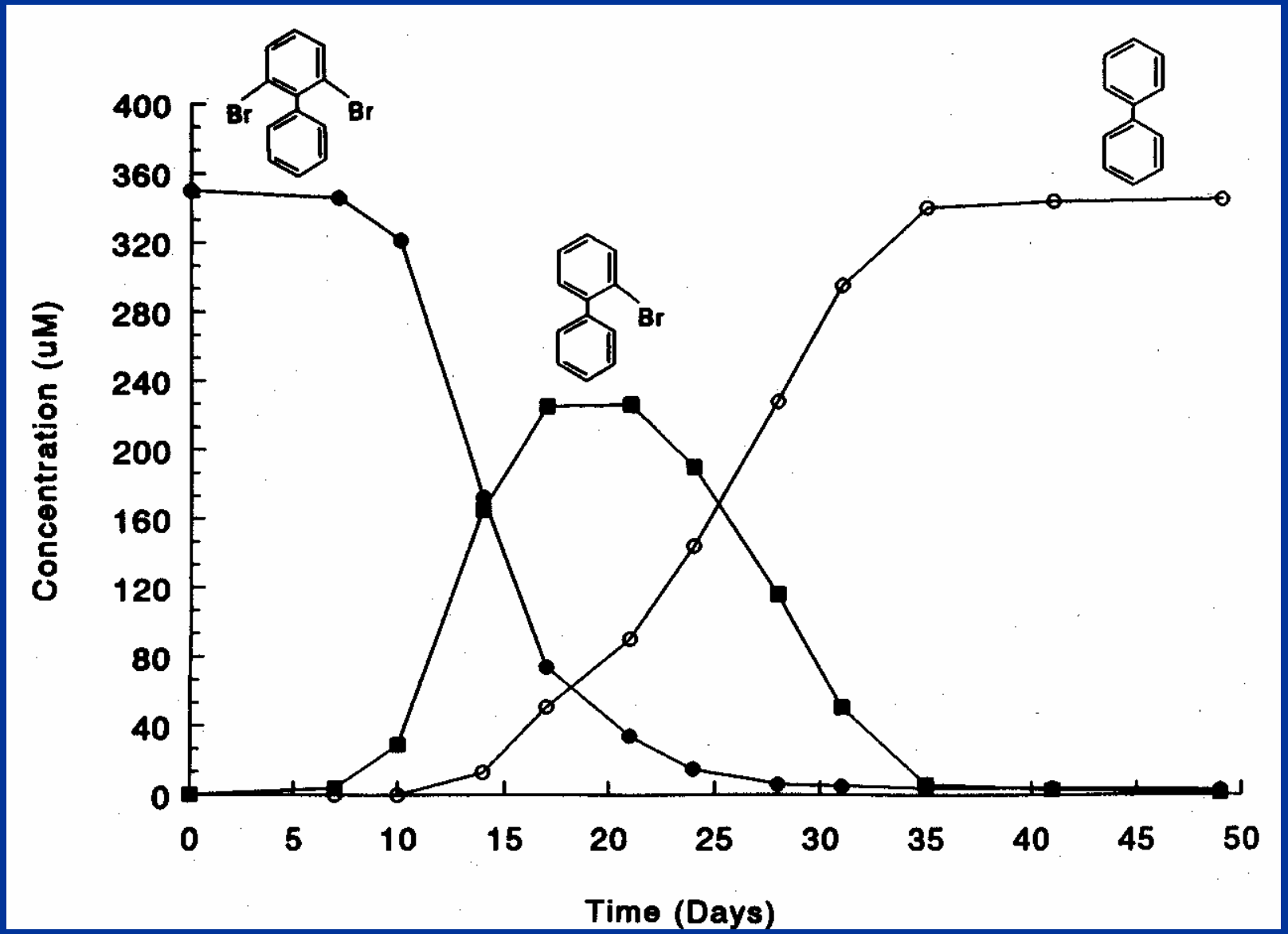
Hypothesis

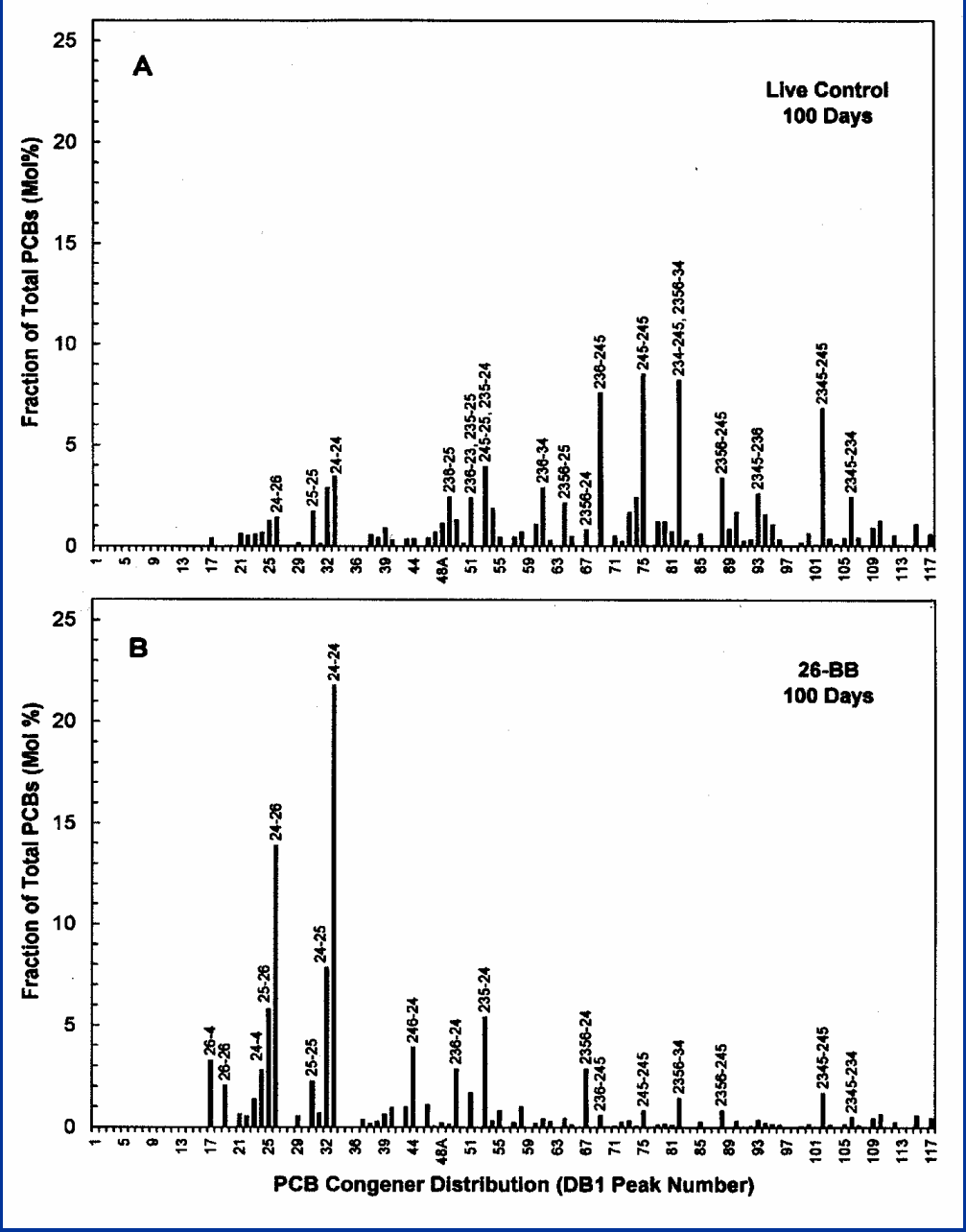
- PCB dechlorinators use halogenated biphenyls as electron acceptors

Corollary

- Halogenated biphenyls can be used to selectively enrich PCB-dechlorinating bacteria

Time Course of Dehalogenation of 26-BB





Effect of Process N Dechlorination on PCB Congener Distribution

Priming PCB Dechlorination

Hypothesis

- PCB dechlorinators use halogenated biphenyls as electron acceptors

Corollary

- Halogenated biphenyls can be used to selectively enrich PCB-dechlorinating bacteria

Result

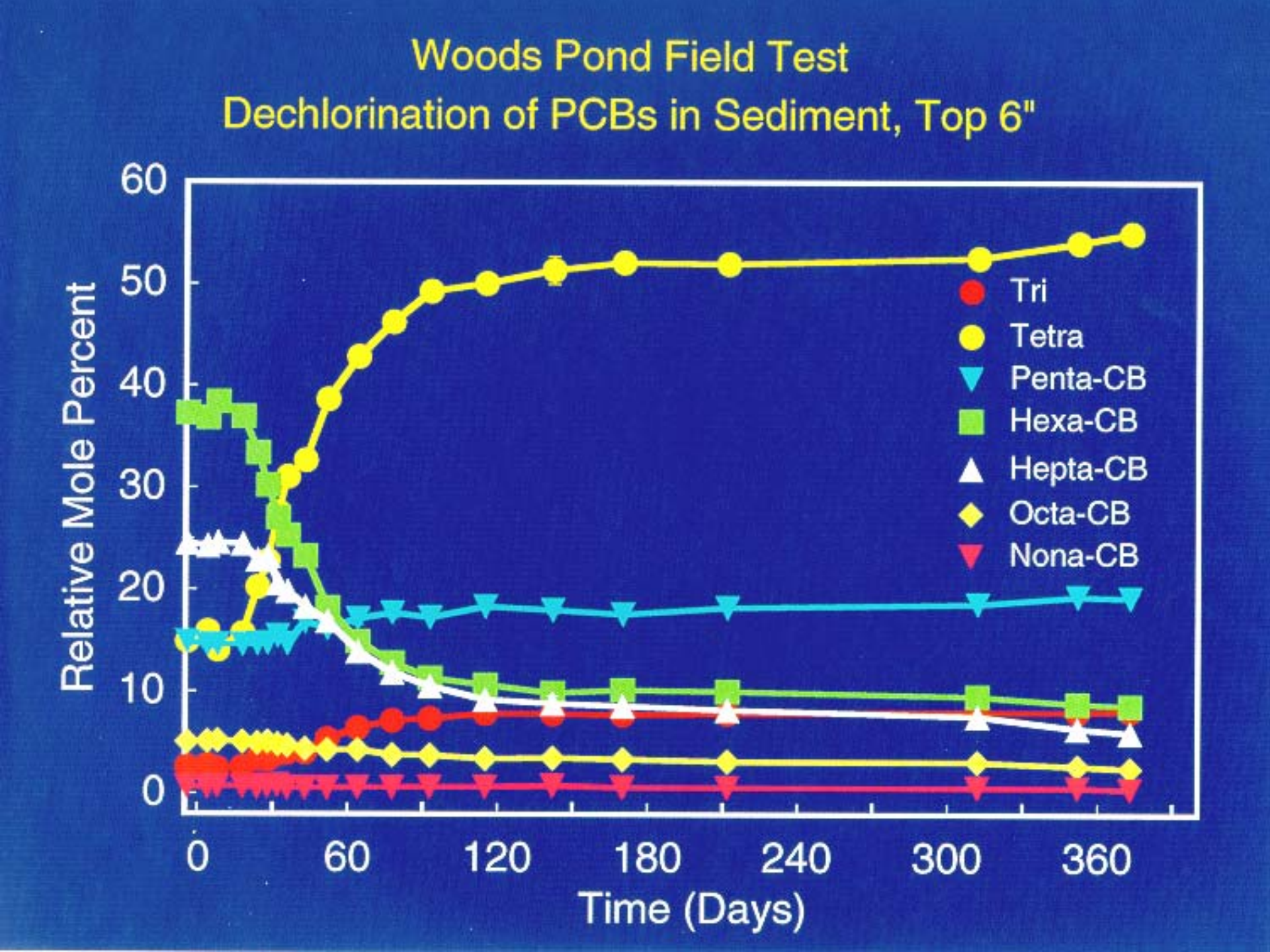
- 26-BB $\rightarrow$ 1000 fold increase in PCB dechlorinators (from 10^5 to 10^8 cells/ml)
- Increased numbers of PCB dechlorinators dechlorinate decades old PCBs in sediment

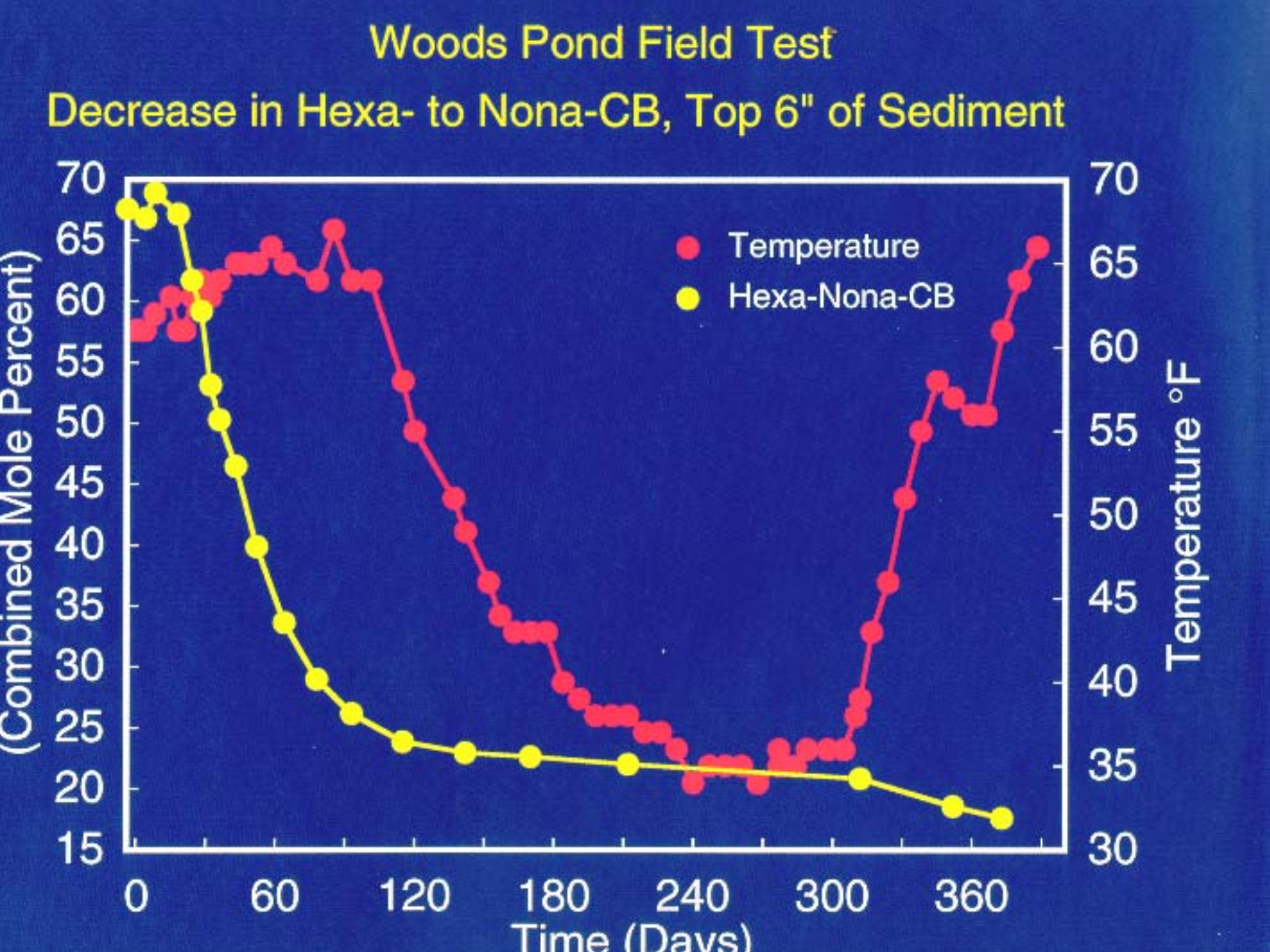
Field Test Parameters

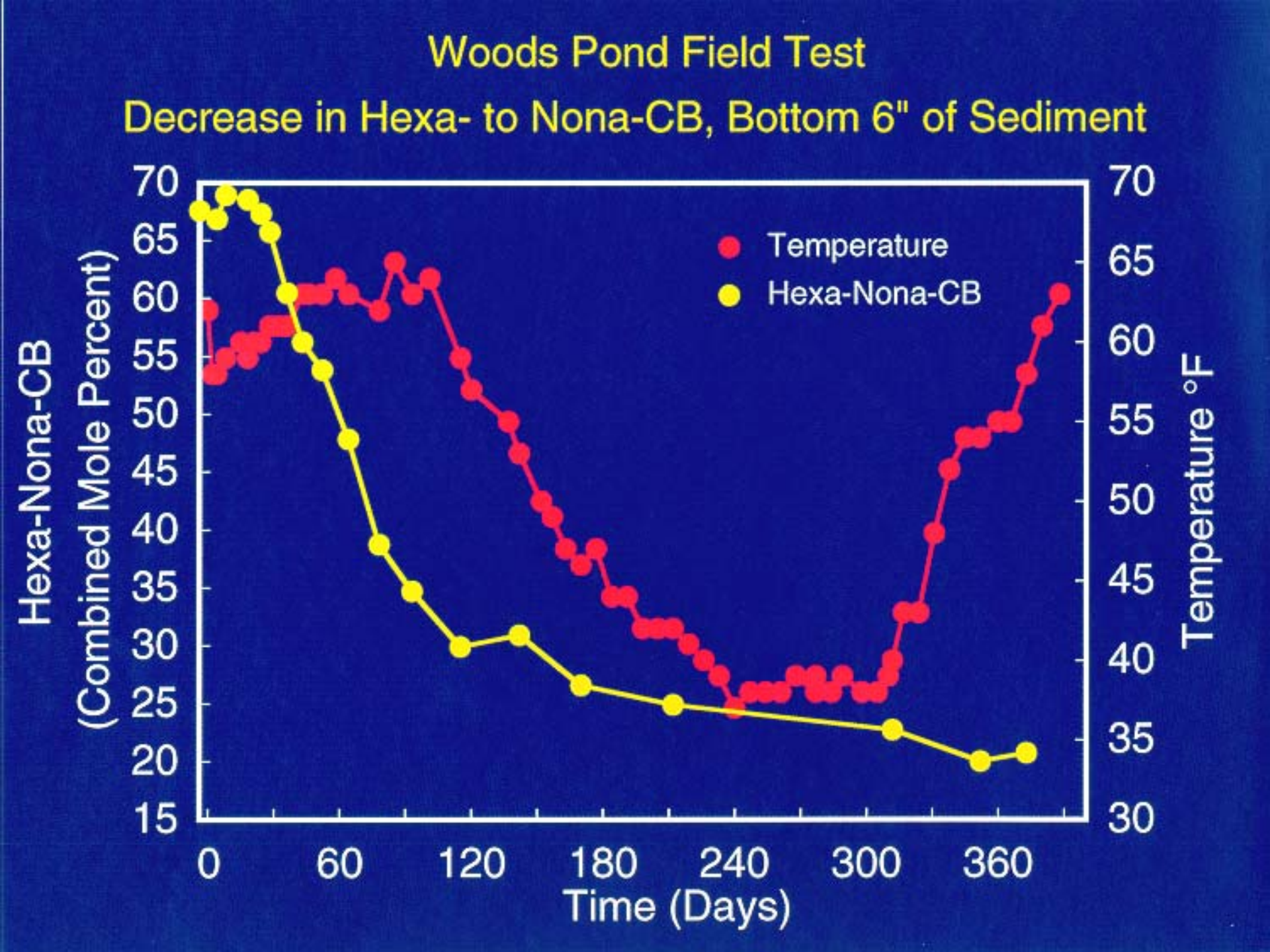
- 400 Kg Sediment (Dry weight)
- 3500 L Water and Sediment
- PCBs 26 ppm (dry wt)

Amendments

- 2,6-dibromobiphenyl 350 μM (880 ppm)
- 17 L Acetone (0.5%)
- Disodium malate (10 mM)


Woods Pond Field Test
Dechlorination of PCBs in Sediment, Top 6"
Relative Mole Percent
60
50
40
30
20
10
0
0
60
120
180
240
300
360
Time (Days)
Tri
Tetra
Penta-CB
Hexa-CB
Hepta-CB
Octa-CB
Nona-CB


Woods Pond Field Test
Decrease in Hexa- to Nona-CB, Top 6" of Sediment
(Combined Mole Percent)
70
65
60
55
50
45
40
35
30
25
20
15
Temperature
Hexa-Nona-CB
Temperature °F
70
65
60
55
50
45
40
35
30
0
60
120
180
240
300
360
Time (Days)


Woods Pond Field Test
Decrease in Hexa- to Nona-CB, Bottom 6" of Sediment
Hexa-Nona-CB
(Combined Mole Percent)
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30
25
20
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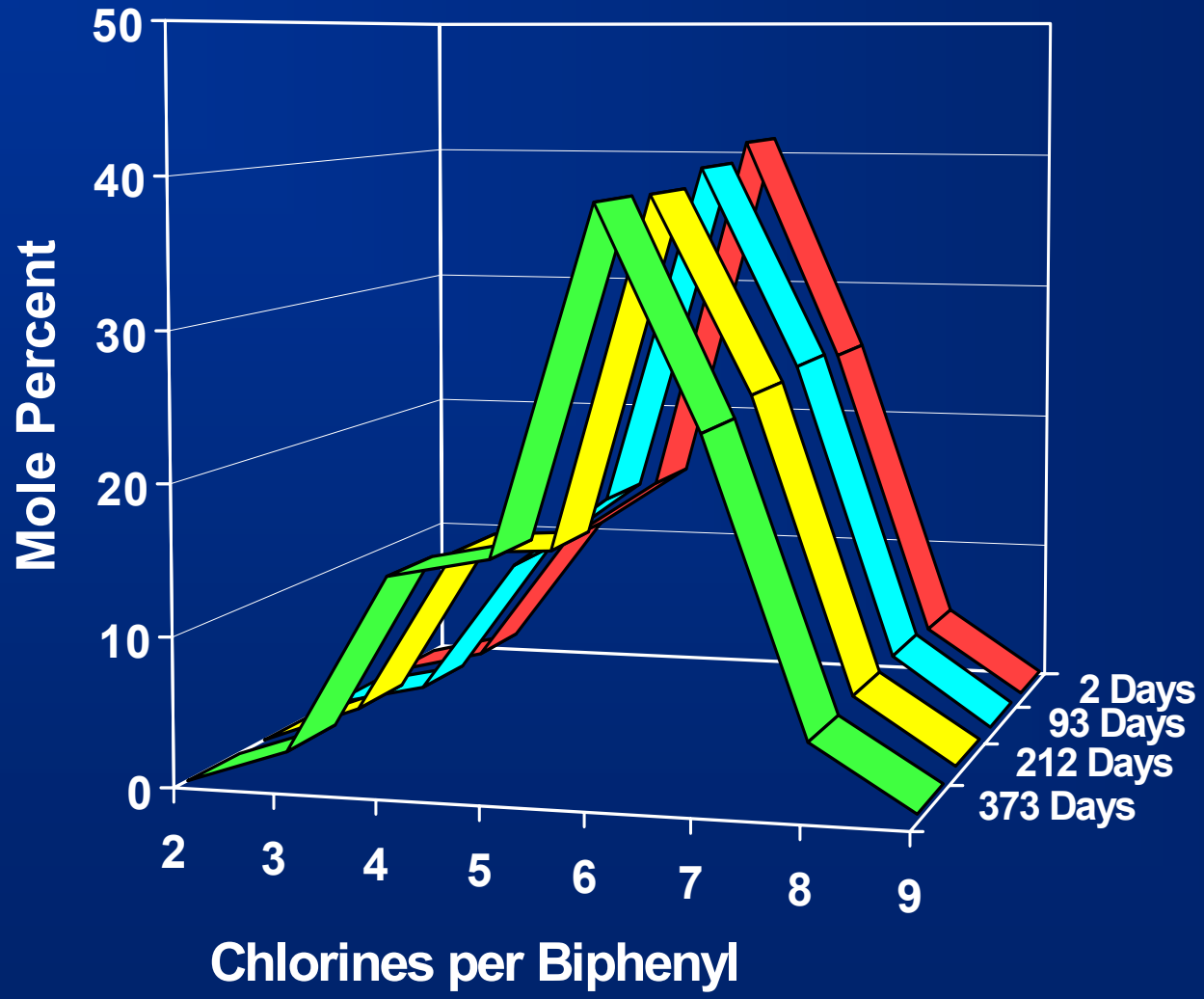
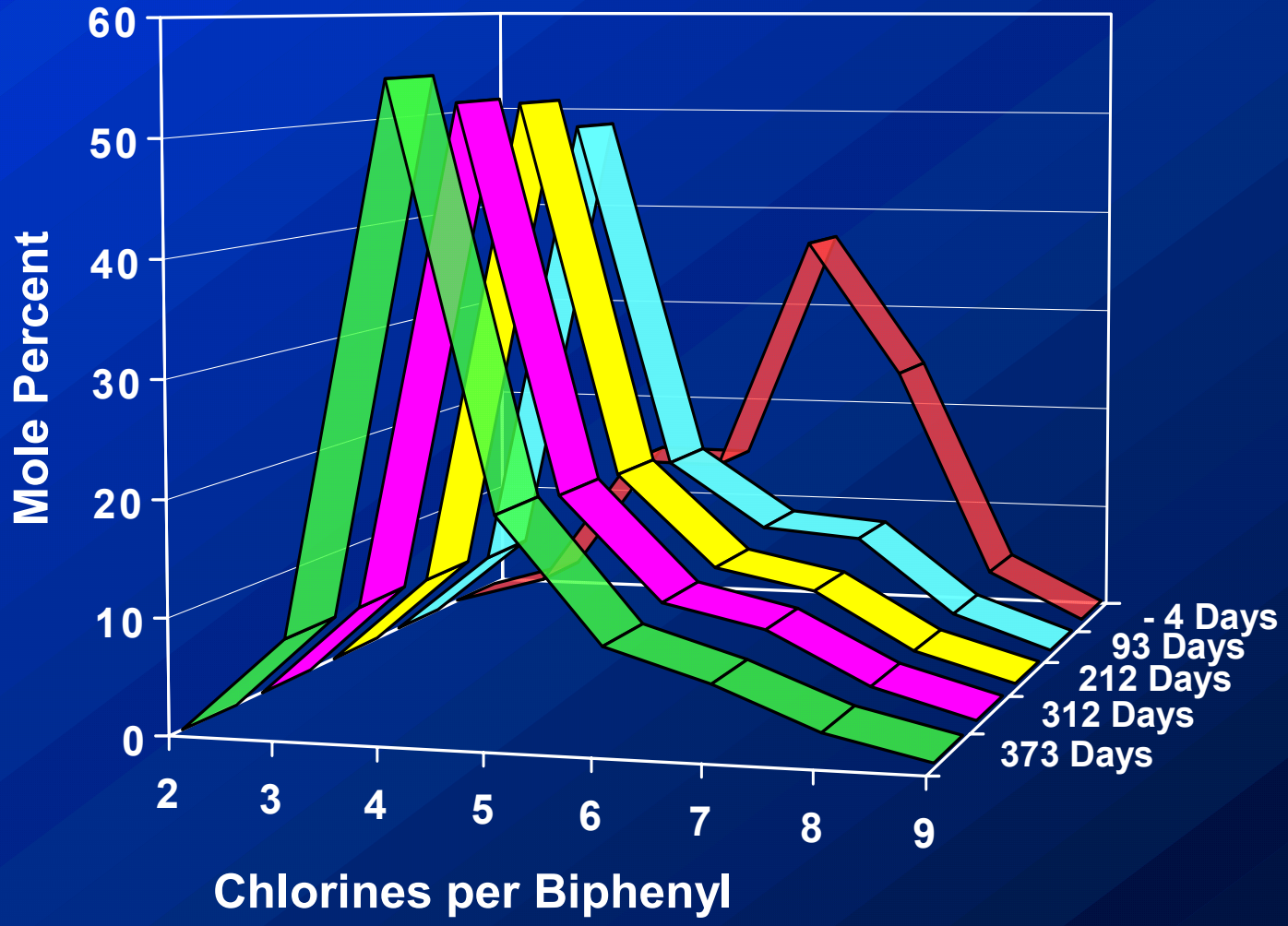
Temperature
Hexa-Nona-CB
Temperature °F
70
65
60
55
50
45
40
35
30
0
60
120
180
240
300
360
Time (Days)

PCB Homolog Distribution: Control Caisson



PCB Homolog Distribution: Experimental Caisson



Field Test Results

Day	Hexa- to Nona-CBs	
	Mol %	% Decrease
0	68	
93	26	62
212	22	68
373	17.6	74

Field Test Results

Homolog	Initial	% Decrease	
	Mol %	93 Days	373 Days
Hexa-CB	38	70	77
Hepta-CB	24	57	75
Octa-CB		27	50

Strategy for Enriching/Isolating PCB Dechlorinating Bacteria

- Exploit specific temperature, primer, and pH requirements
- Use inhibitors and antibiotics to inhibit bacteria not essential to the PCB-dechlorinating community
- Repeatedly transfer with primers onto sterile PCB-contaminated sediment and then defined medium
- Use molecular tools to monitor the microbial population and identify key microorganisms

Recent Findings

- Putative PCB dechlorinators in Woods Pond are ***Dehalococcoides*** spp.
- Grow on defined medium while retaining full PCB-dechlorinating activity

- ***Dehalococcoides*** genome reveals 17 reductive dehalogenase homologs (TIGR)
- Dehalorespiration is only known means of growth
- ***Dehalococcoides*** substrates:

  Dhc sp. CBDB1: Hexachlorobenzene, 1,2,3,7,8-PeCDD (Lorenz Adrian and colleagues)

  Dhc ethenogenes : Hexachlorobenzene, 1,2,3,4-TeCDD (Steve Zinder and Donna Fennel)

Evaluation of the Technology

Strengths

- Natural process
- Effective *in situ* at 12 to 30°C
- May be able to grow dechlorinators in lab and bioaugment without adding a primer
- Can reduce toxicity & persistence

Weaknesses

- Success depends on microbial composition
- Affected by organic content and xenobiotics
- Requires addition of a halogenated primer
- May not work well for very low concentrations
- Dechlorinates but does not destroy

Acknowledgments

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