Aerobic and Anaerobic Biodegradation of PCBs

Presentation to
EPA Region II

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New York City, NY
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Aerobic Degradation

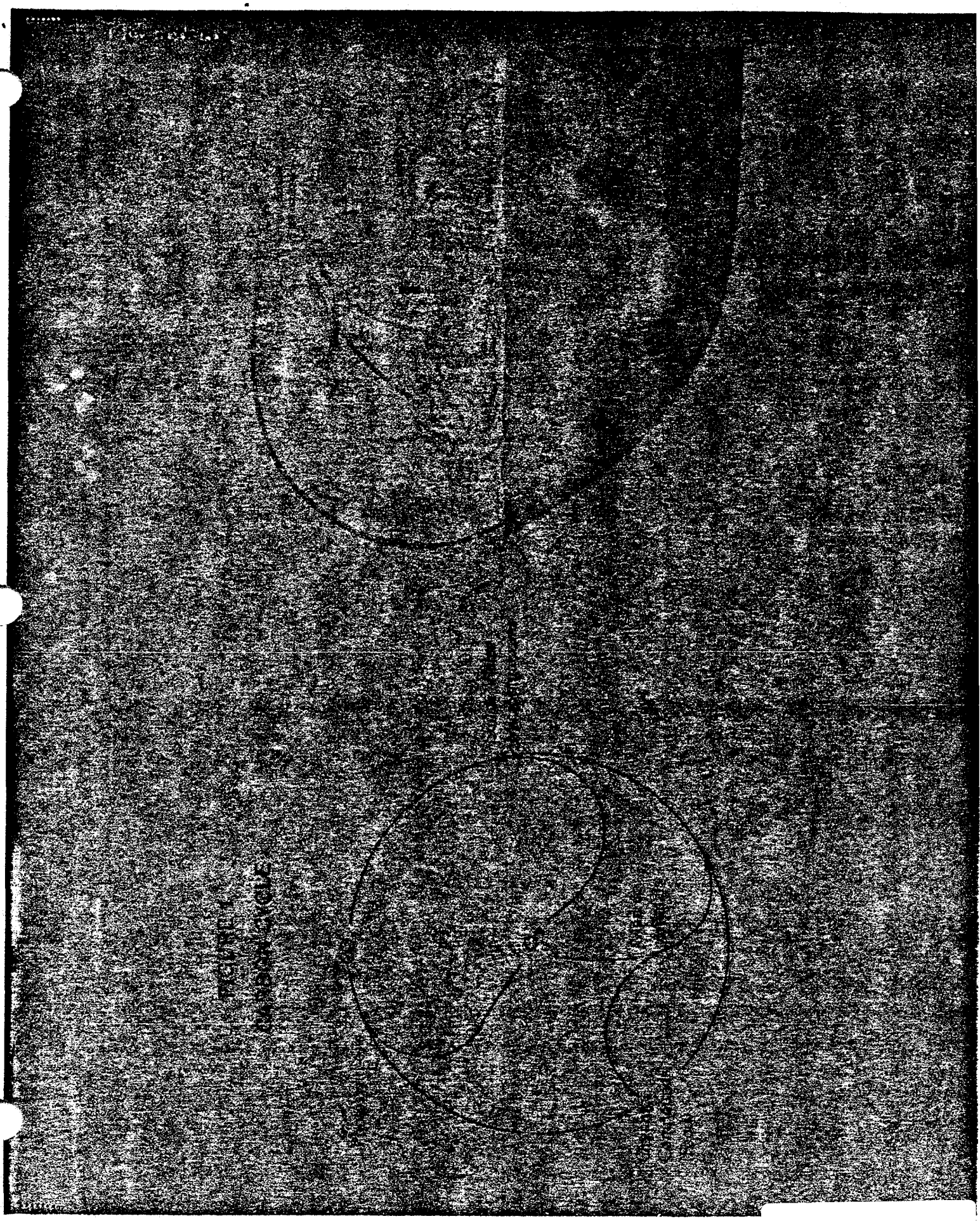
- Introduction
- rDNA Efforts
- Dragstrip

Anaerobic Dechlorination

- Introduction
- Aroclors
- Single Congener

Anaerobic/Aerobic

- Lab Results



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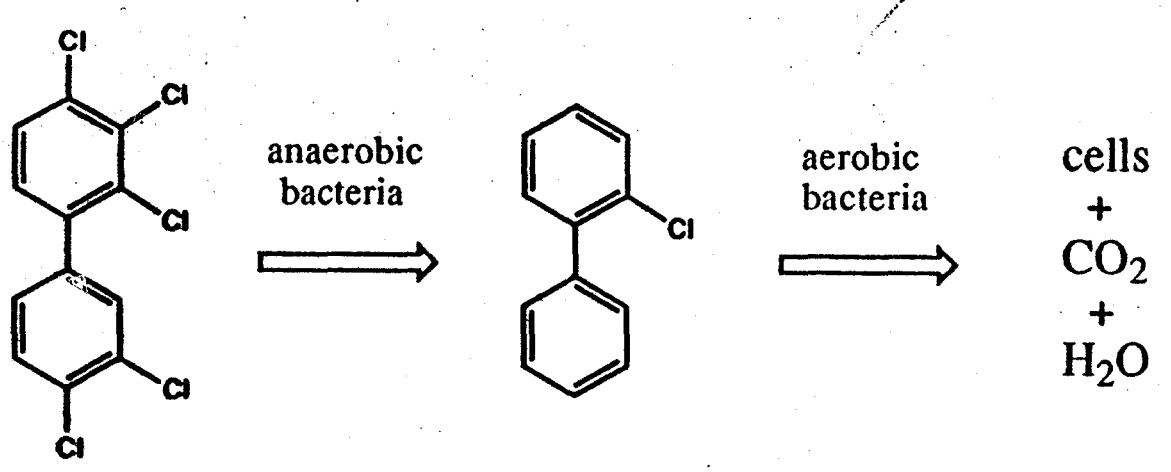
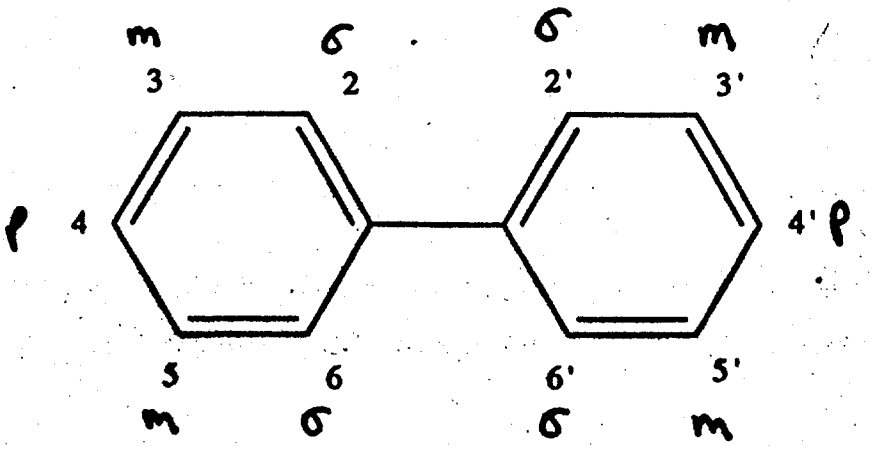
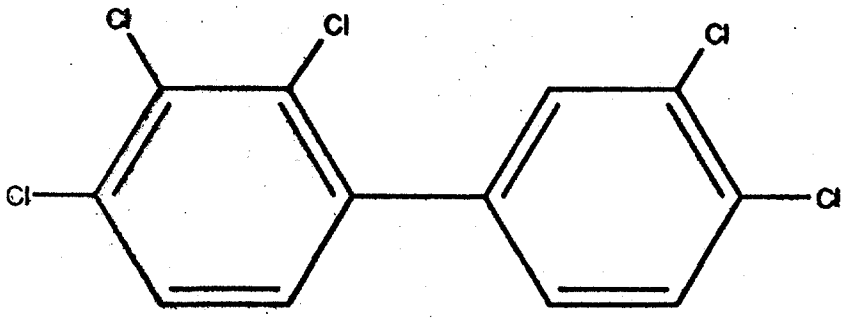

Cl
Cl
Cl
Cl
Cl
anaerobic
bacteria
Cl
aerobic
bacteria
cells
+
CO_2
+
H_2O

209 PCBs

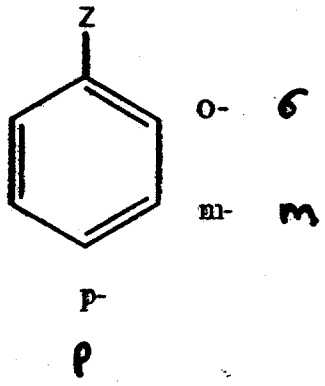


1,1'-biphenyl [95-92-4]



2,3,3',4,4'-pentachloro,1,1'-biphenyl
2,3,3',4,4'-pentachlorobiphenyl
2,3,4,3',4'
234-34

ortho (o-) 1,2-
meta (m-) 1,3-
para (p-) 1,4-



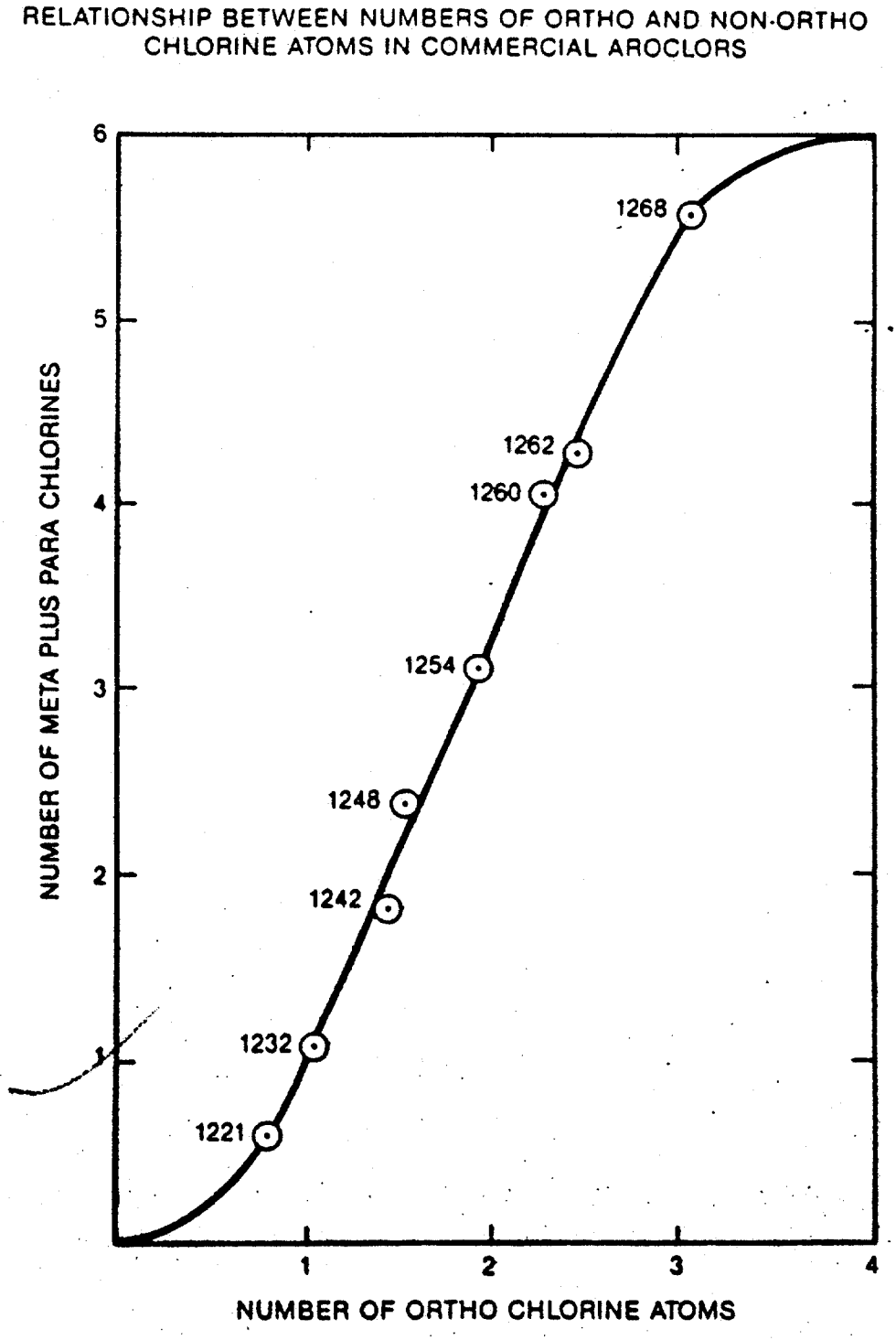
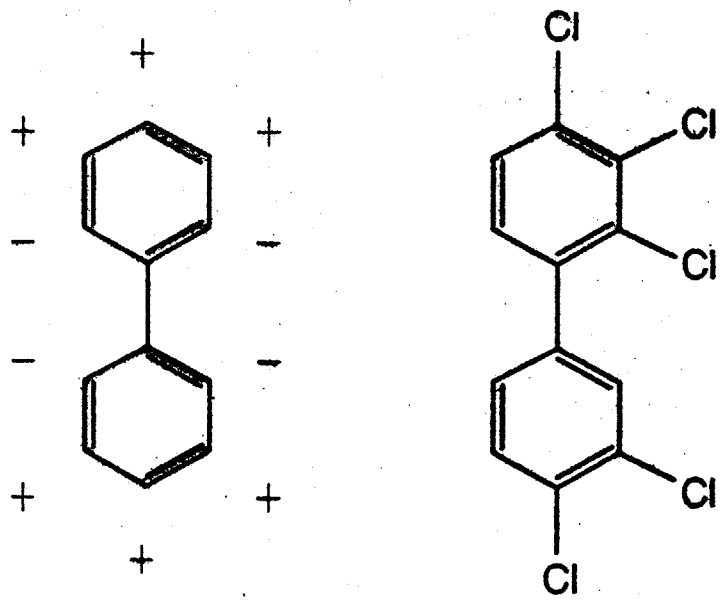


Figure 8-1. Relationship between numbers of *ortho* and non-*ortho* chlorine atoms in commercial Aroclors.

Structure / Activity Relationships

PCB TOXICITY



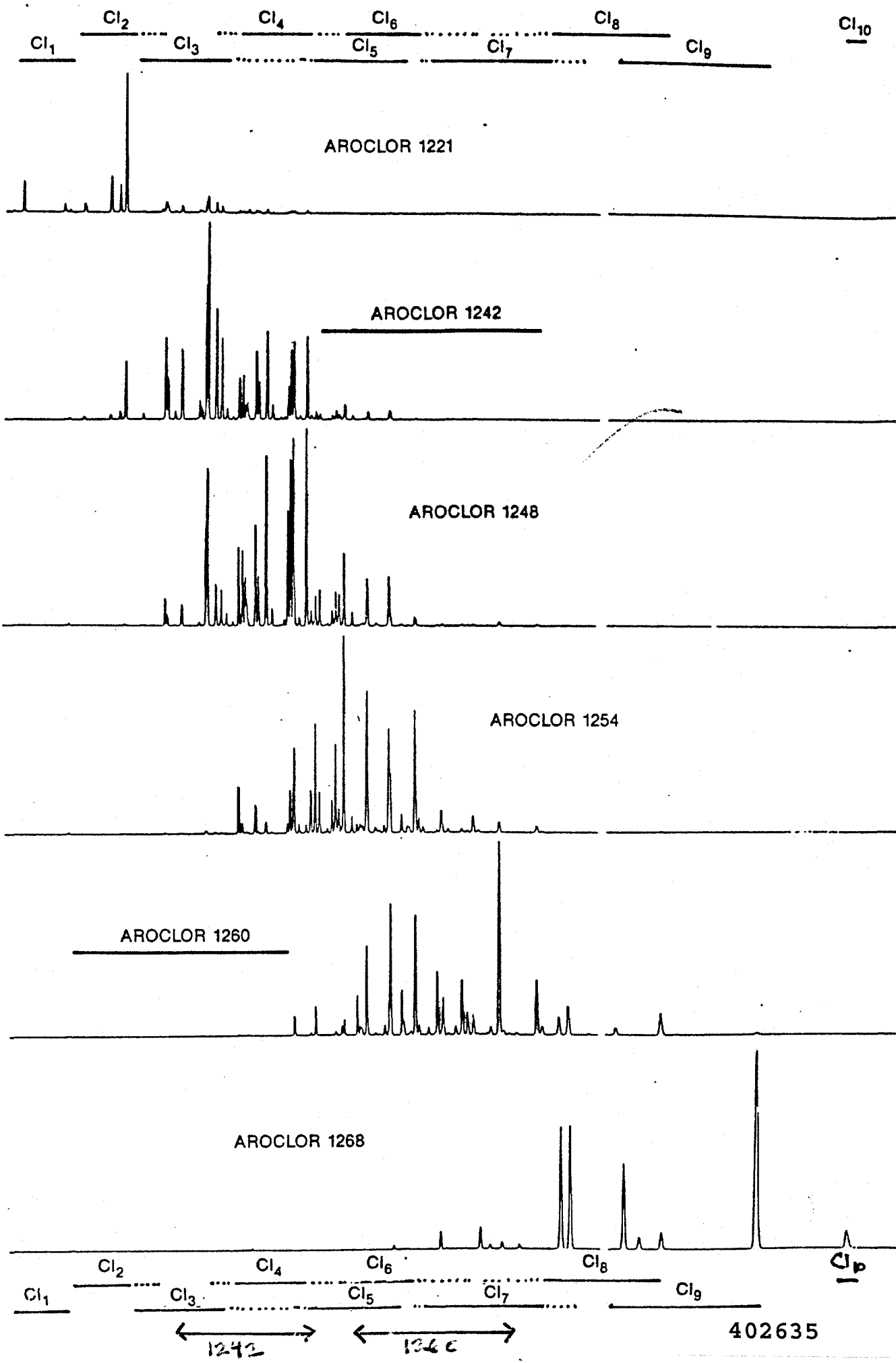
234-34-CB

meta +
para +
ortho −

ADAPTED FROM:

S. Safe et al. (1982) "PCBs: Structure - Activity Relationships," Adv. Exposure, Health, Environ. Eff. Stud. PCBs, Symposium Proc. PB 84-135771, PP 229-248

P.G. Olafsson et al. (1987) "PCB Congener - Specific Analysis: A Critical Evaluation of Toxic Levels in Biota," Chemosphere 16, 2585-2593.


Cl2
Cl4
Cl6
Cl8
Cl10
Cl1
Cl3
Cl5
Cl7
Cl9
AROCLOR 1221
AROCLOR 1242
AROCLOR 1248
AROCLOR 1254
AROCLOR 1260
AROCLOR 1268
Cl2
Cl4
Cl6
Cl8
Cl10
Cl1
Cl3
Cl5
Cl7
Cl9
1242
1260
402635

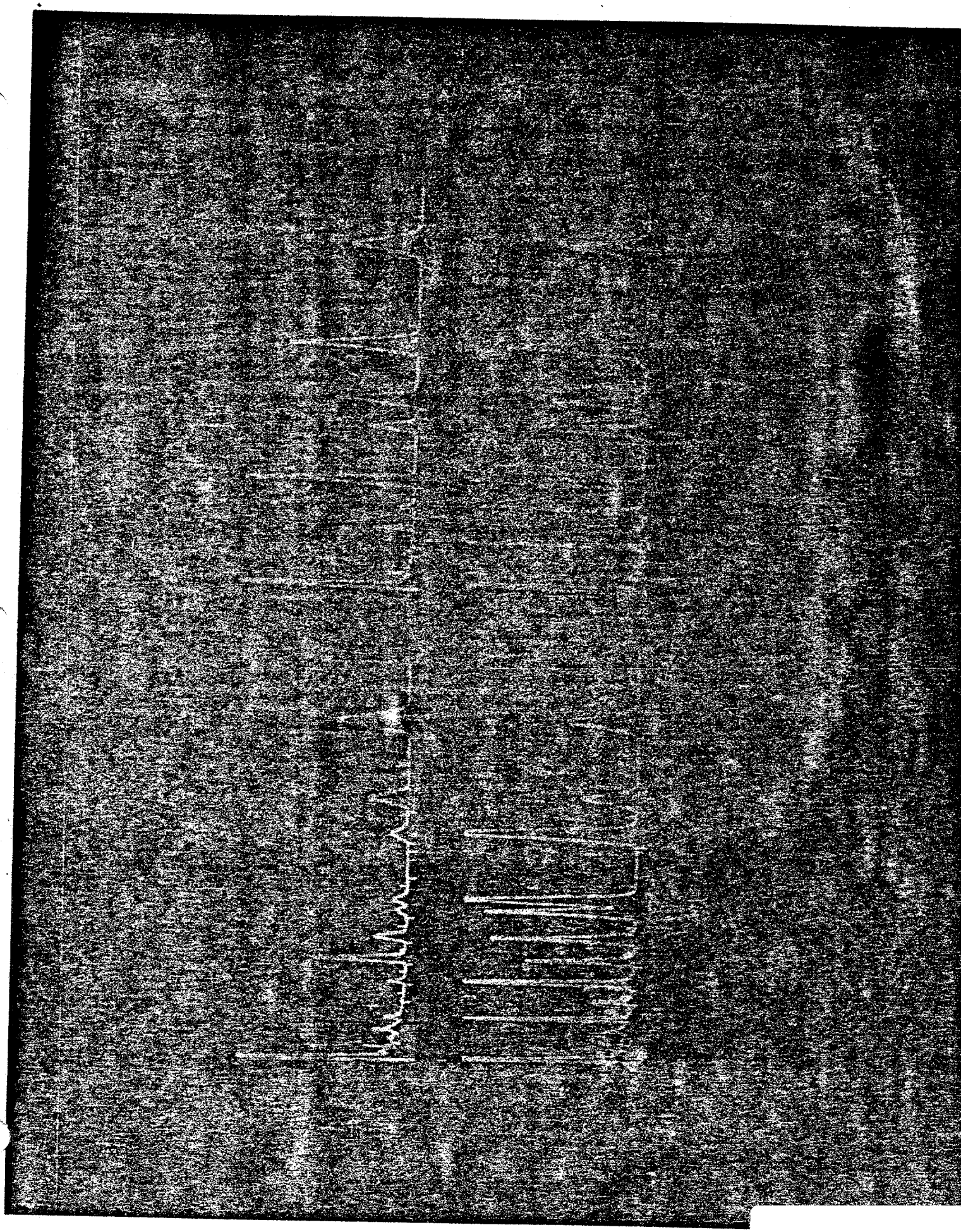


Table 1: Identification of PCB-degrading environmental isolates

Strain designation	Organism
MB1	*Corynebacterium* sp.
Pi434	*Alcaligenes faecalis*
H850	*Alcaligenes eutrophus*
H201, Pi704, RJB	*Pseudomonas cepacia*
H128, H336, H430	*Pseudomonas testosteroni*
LB400, LB410	*Pseudomonas* sp.
Pi939, H1130, Pi304 H702, Pi101	*Pseudomonas* (*Acidovorans* group)

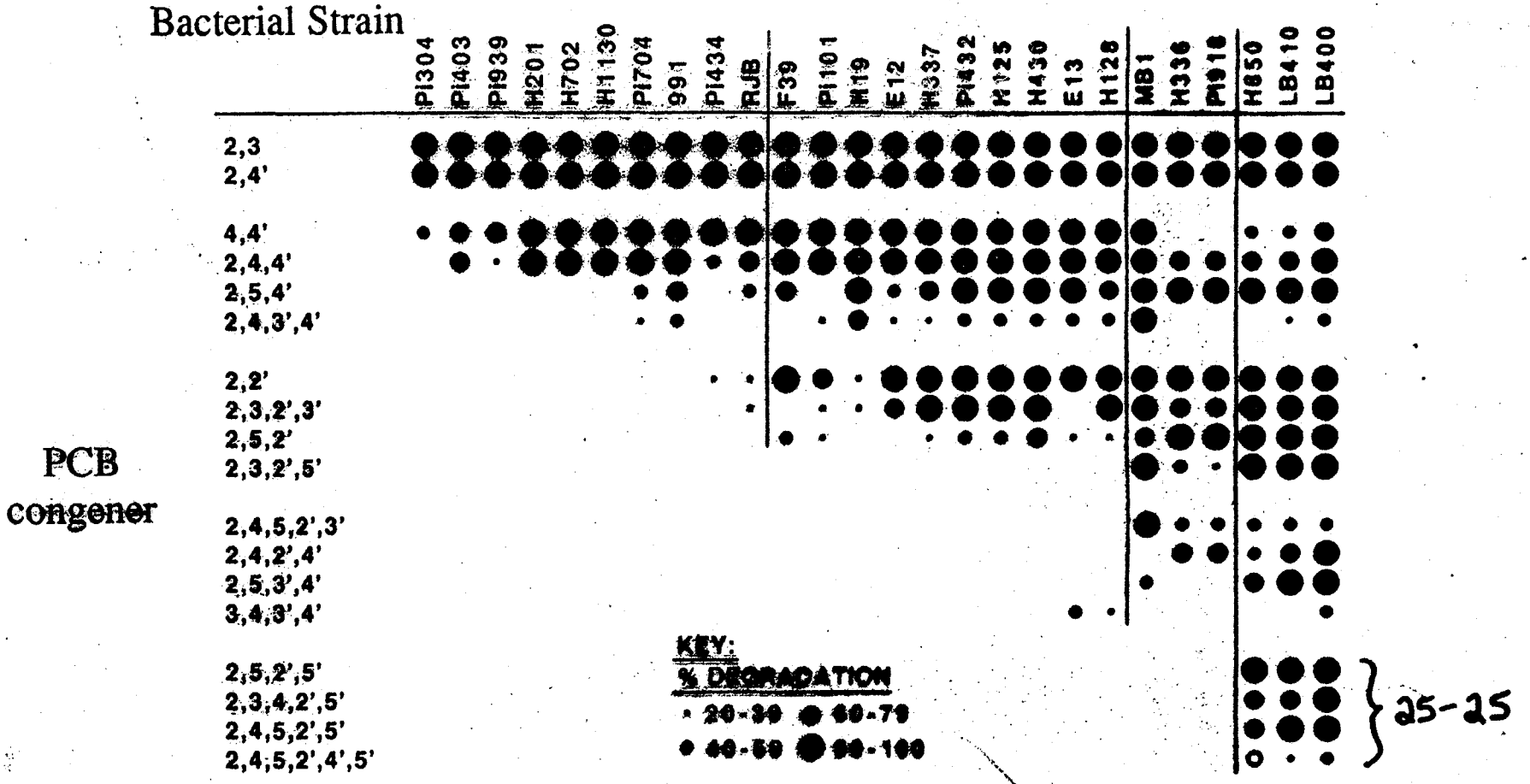
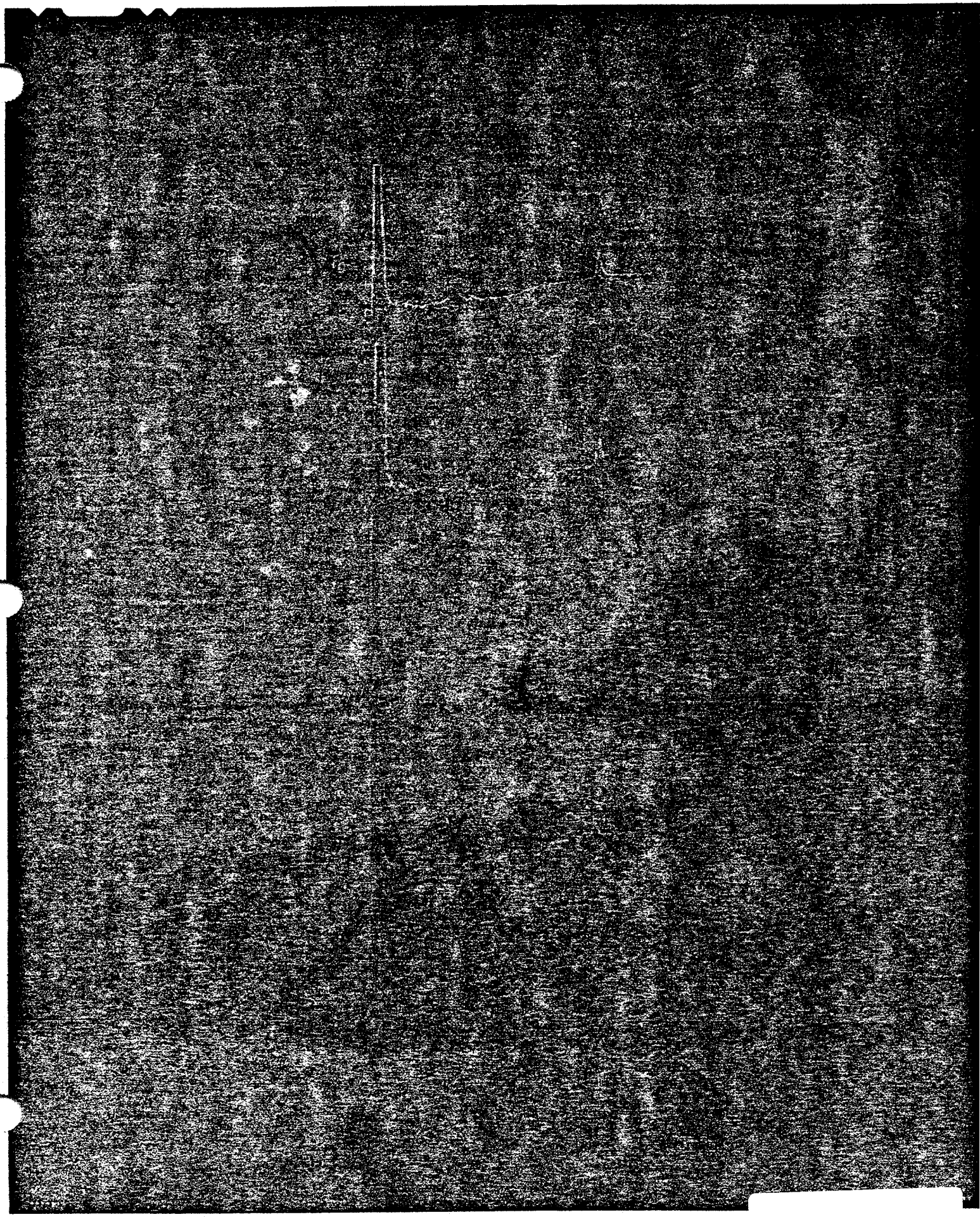
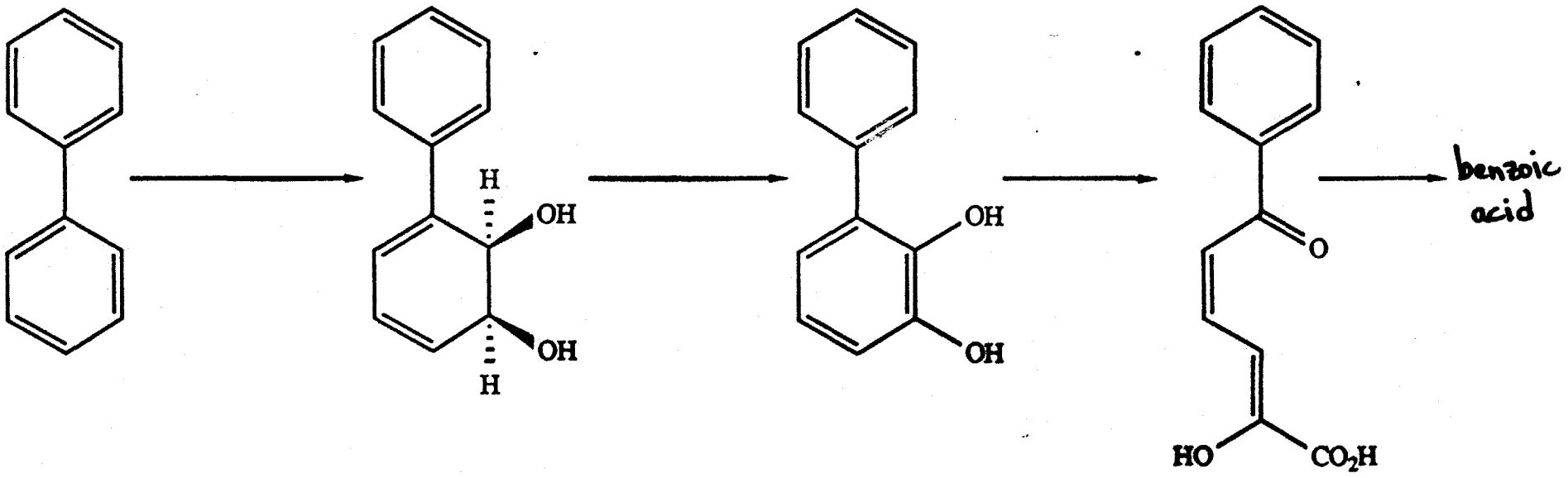


Figure 1: Comparison of the PCB-degrading competence of environmental bacterial isolates. [o] indicates that H850 degraded less than 20% of this congener (245-245-CB), but a metabolite was isolated. [adapted from reference 11]



Biphenyl and PCB Biodegradation Pathway



2,3 biphenyl dioxygenase pathway

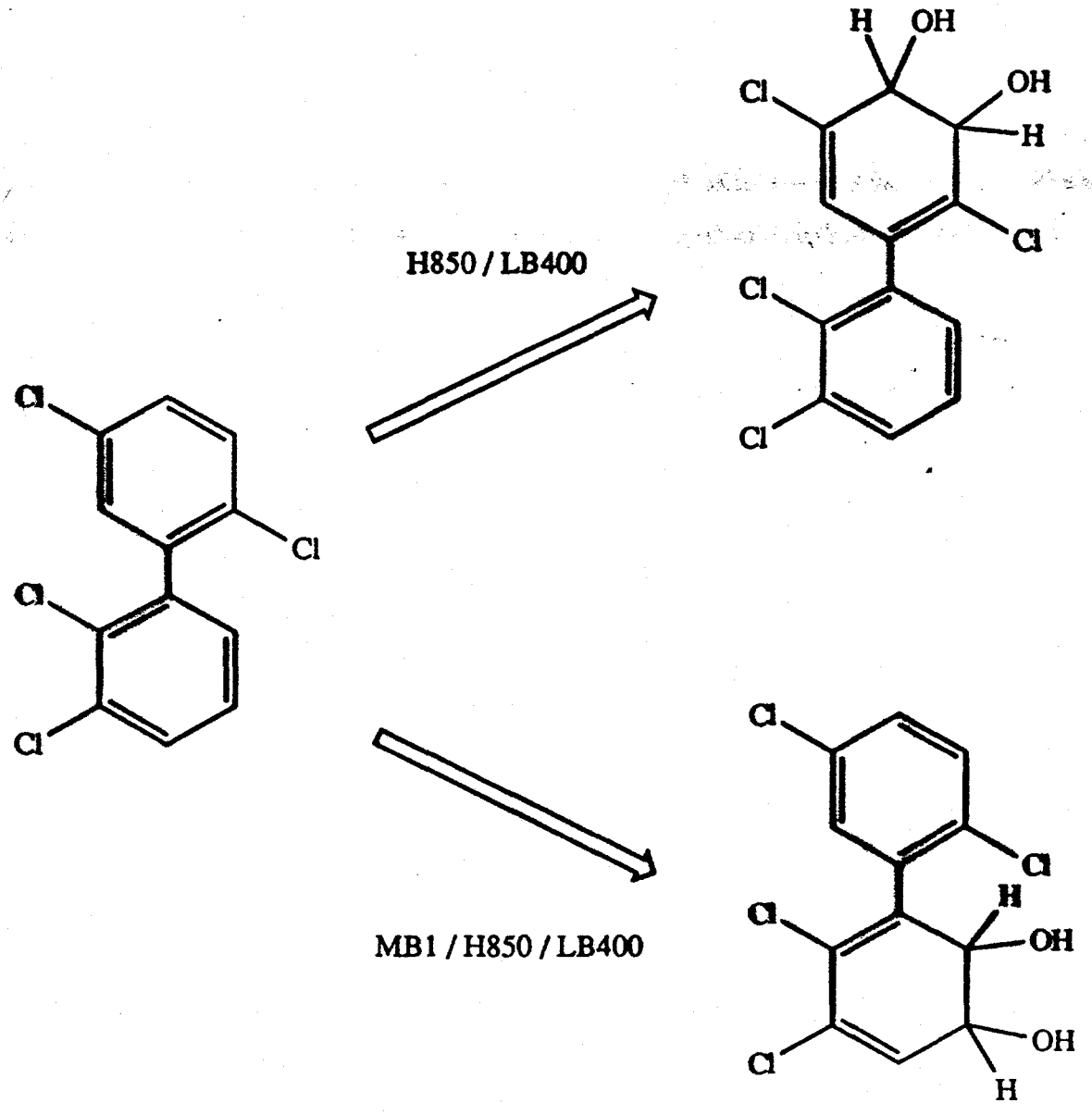


Figure 3: Initial oxidative degradation products for attack via 2,3- and 3,4- dioxygenases on the congener 2,3,2',5'-tetrachlorobiphenyl.

- Aroclor 1242 on soil
- Additive

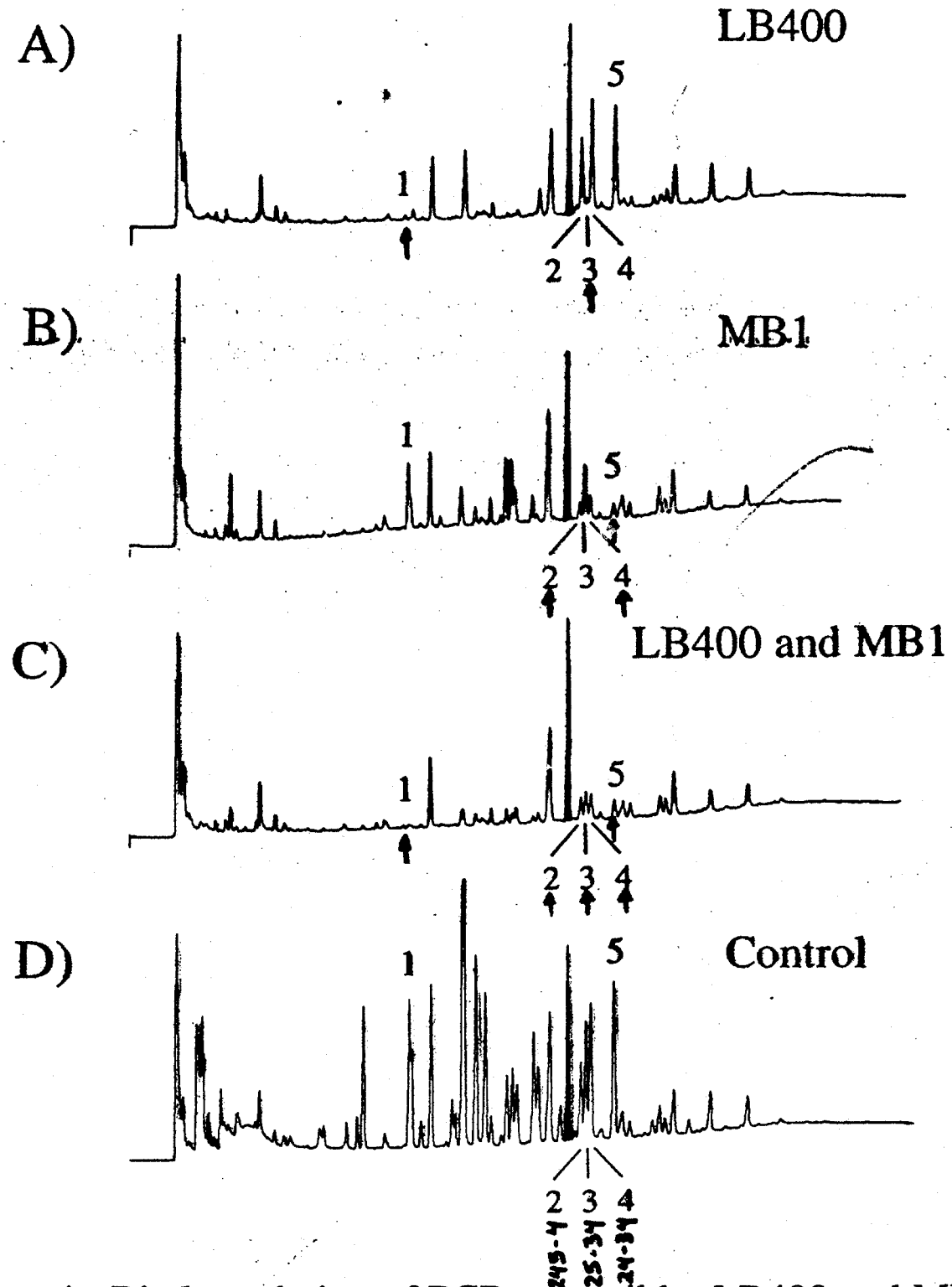


Figure 4: Biodegradation of PCBs on soil by LB400 and MB1 (50ppm Aroclor 1242, 10^9 cells/0.4 gm soil) Shaded peak is not degraded (246-24-CB) and is used as an internal standard. [adapted from reference 4]

MB1 4-4
LB400 25-

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Restriction Enzymes:

TAPE
SCISSORS

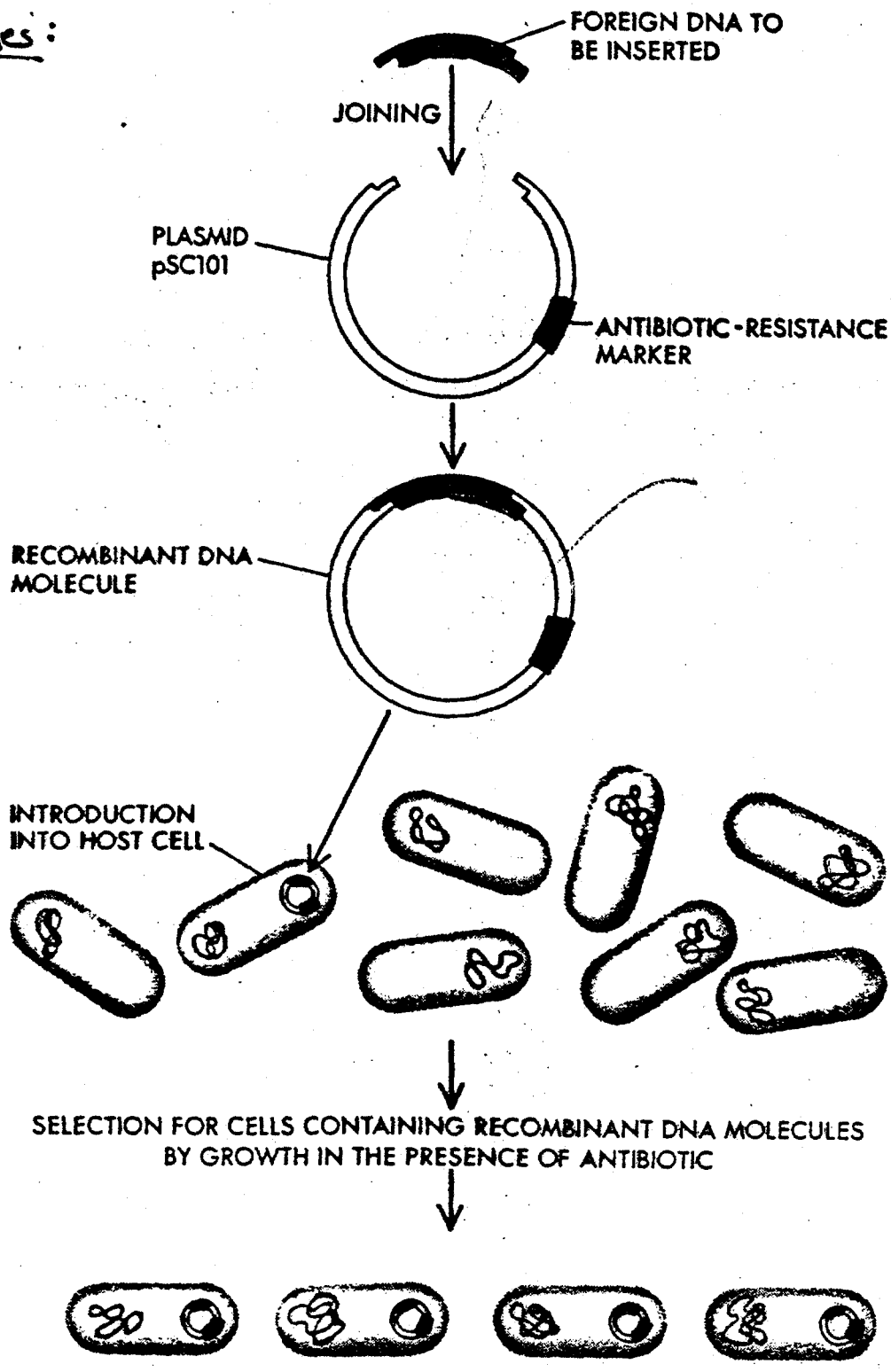


Figure 5-11
The cloning of DNA in a plasmid.

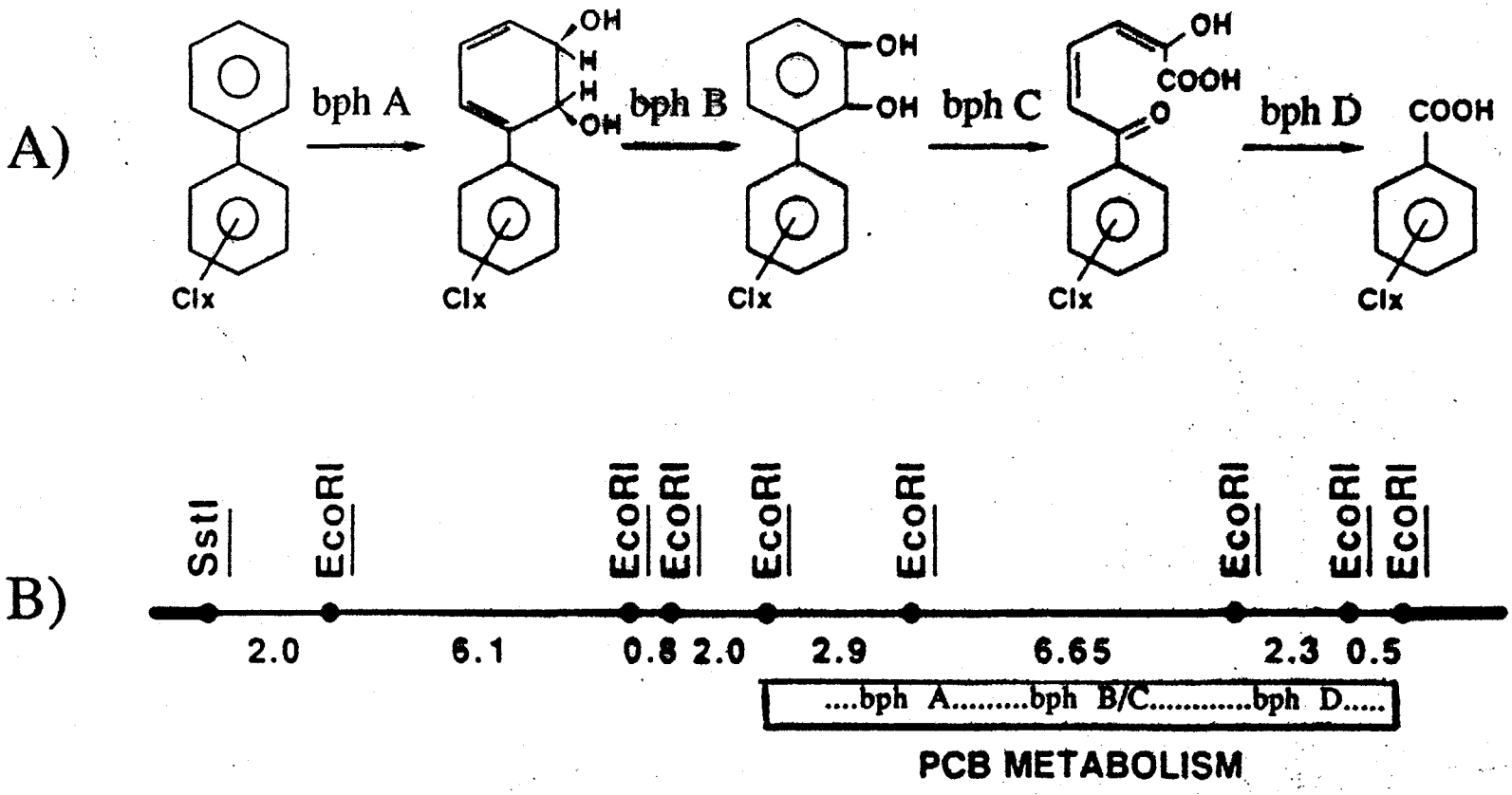


Figure 2: A) Metabolic pathway for biphenyl/PCB degradation
B) Arrangement of the EcoRI fragments in pGEM410
bph A - biphenyl/PCB dioxygenase
bph B - dihydrodiol dehydrogenase
bph C - dihydroxybiphenyl oxygenase
bph D - hydrase

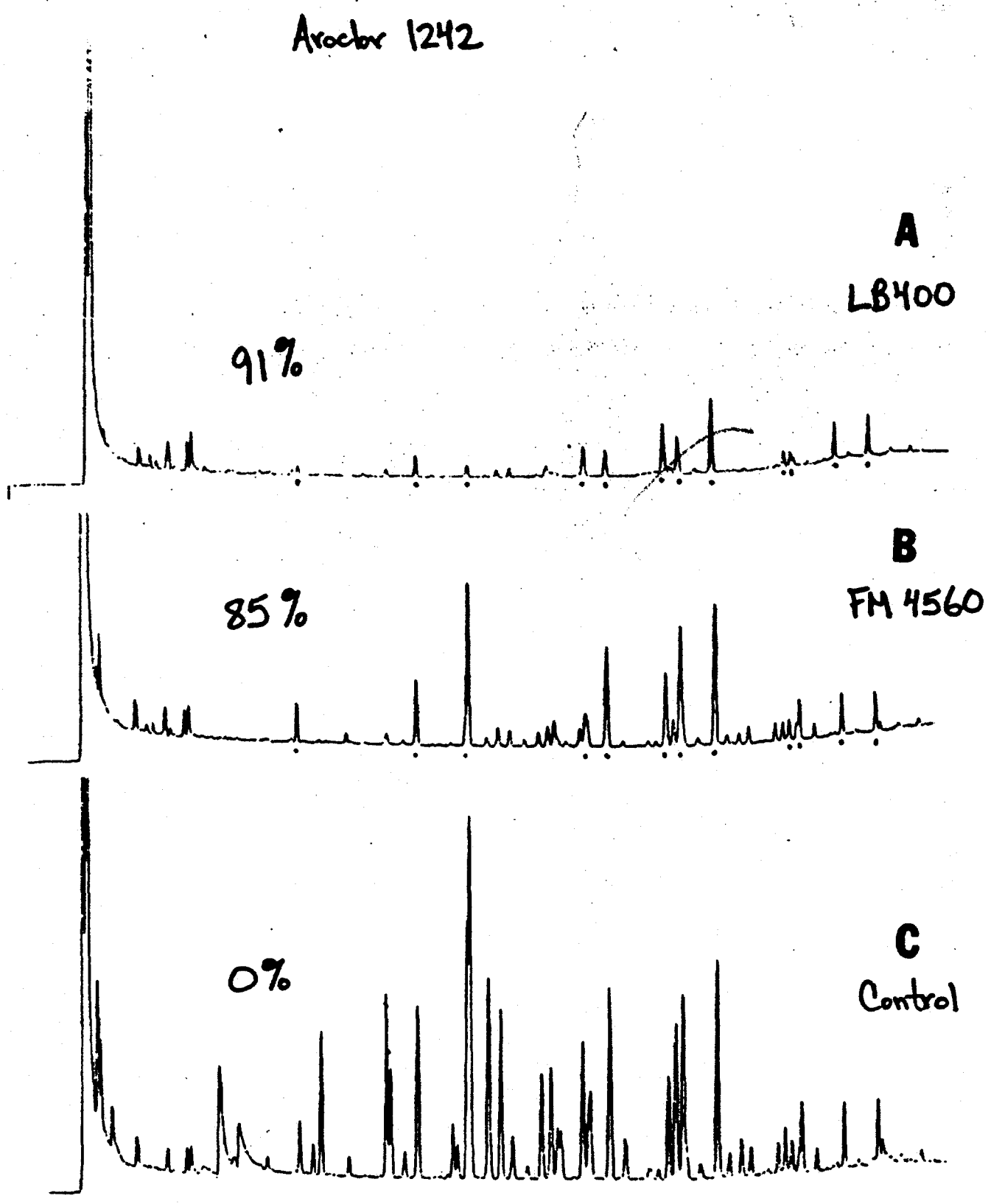


Figure 4-3. **Degradation of Aroclor 1242 (10 ppm) by *Pseudomonas* strain LB400 and *E. coli* FM4560 in a 24-h resting cell assay. Panel A, LB400; panel B, FM4560; panel C, mercury-killed control.**

Table 4-2

PCB-DEGRADATIVE COMPETENCE

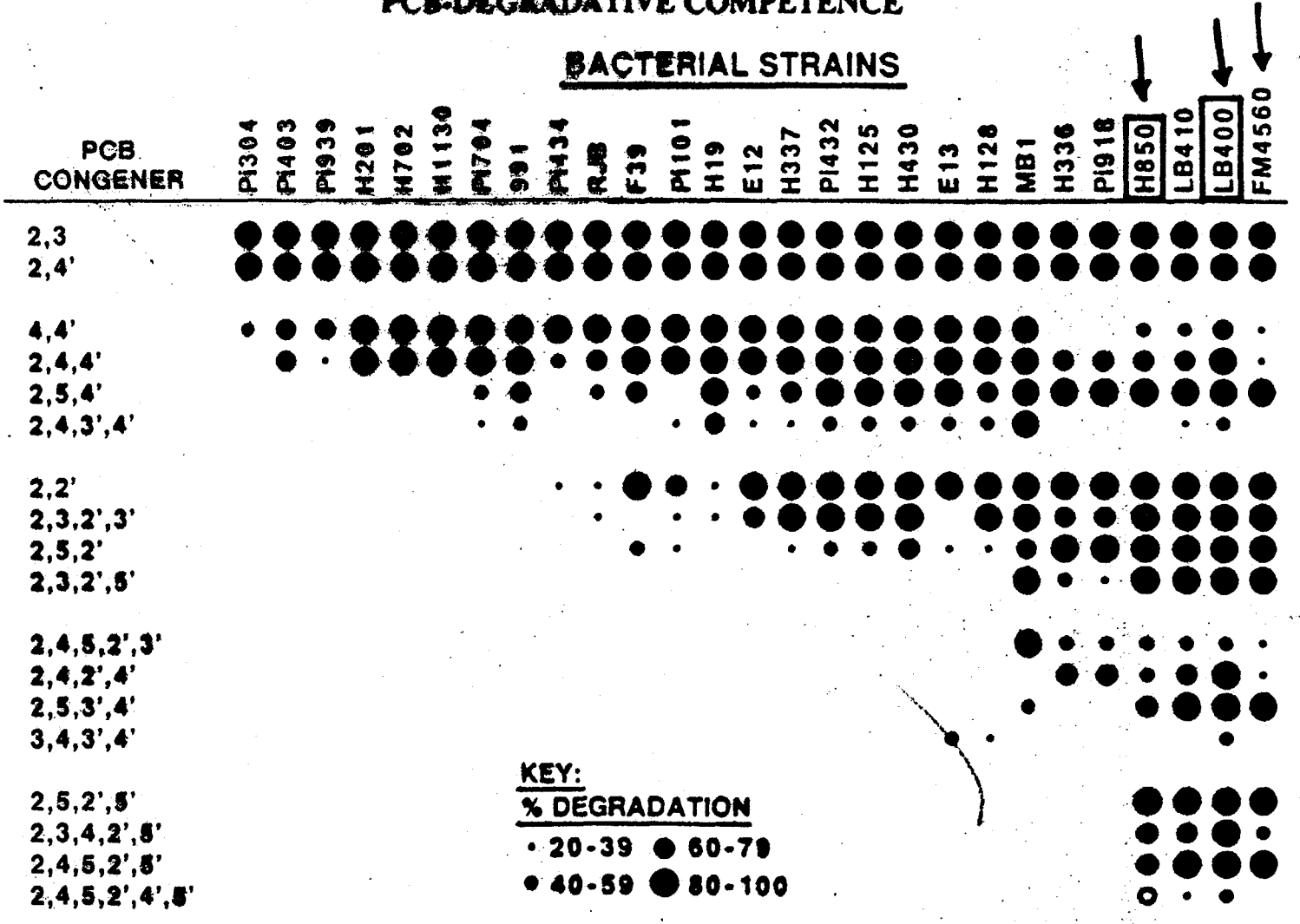


Table 2: Relative PCB degrading activity of *E. coli* recombinant strains and *Pseudomonas* strain LB400.

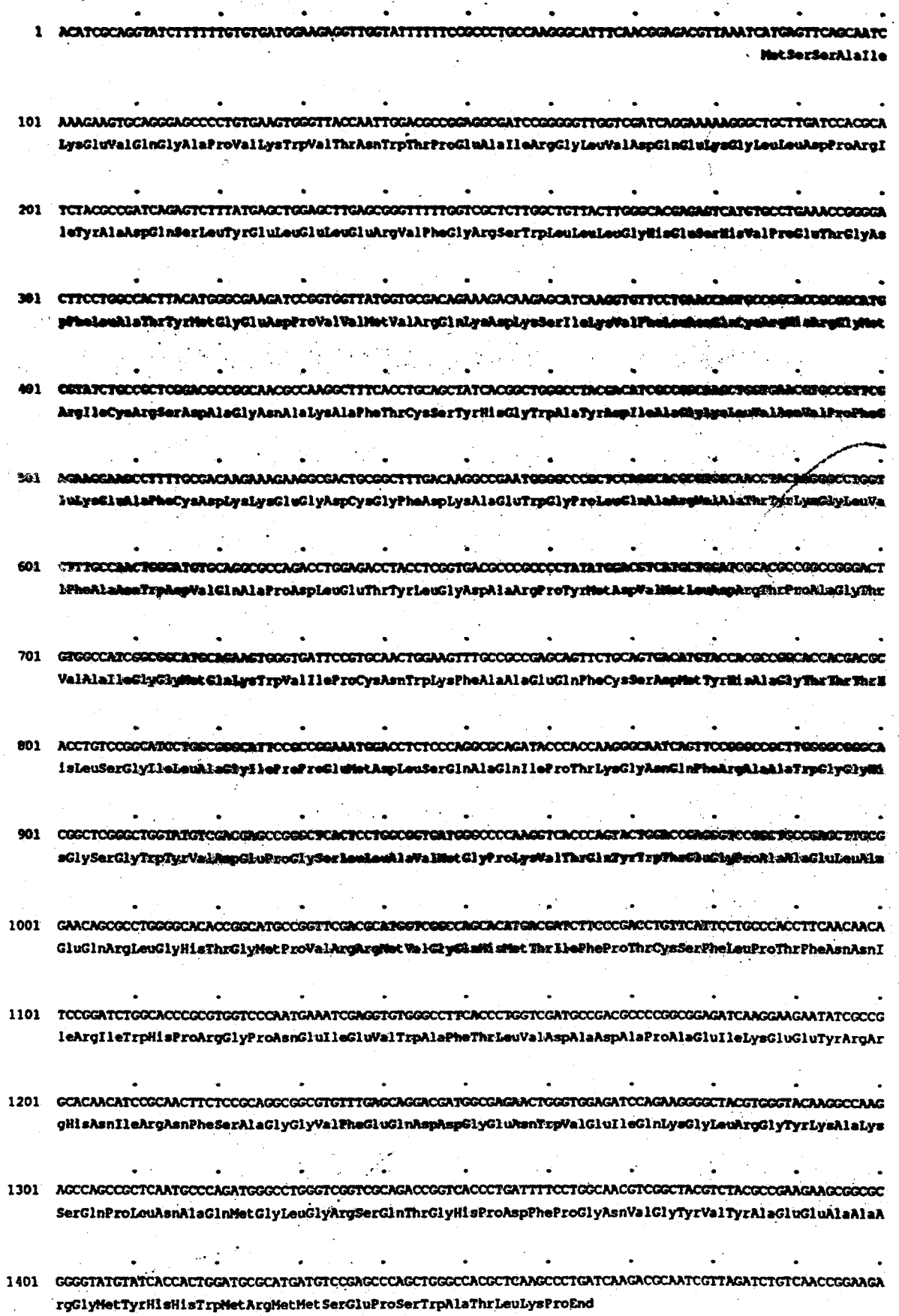
PCB Congener	Percent Activity[a]			
	LB400 (Biphenyl)	FM 4100	FM 4560	LB400 (S)
2,3	●	●	●	●
2,2′	●	●	●	●
2,4′	●	●	●	●
2,5,2′	●	●	●	●
2,5,4′	●	●	●	•
2,3,2′,3′	●	•	●	●
2,3,2′,5′	●	•	●	●
2,5,3′,4′	●	•	●	●
2,5,2′,5′	●	●	●	●
2,4,5,2′,5′	●	o	●	•
2,3,4,2′,5′	●		•	•
2,4,5,2′,3′	●		•	o
4,4′	●		•	
2,4,4′	●	o	●	o
2,4,3′,4′	●		•	
2,4,2′,4′	●		•	
3,4,3′,4′	●			
2,4,5,2′,4′,5′	●		•	

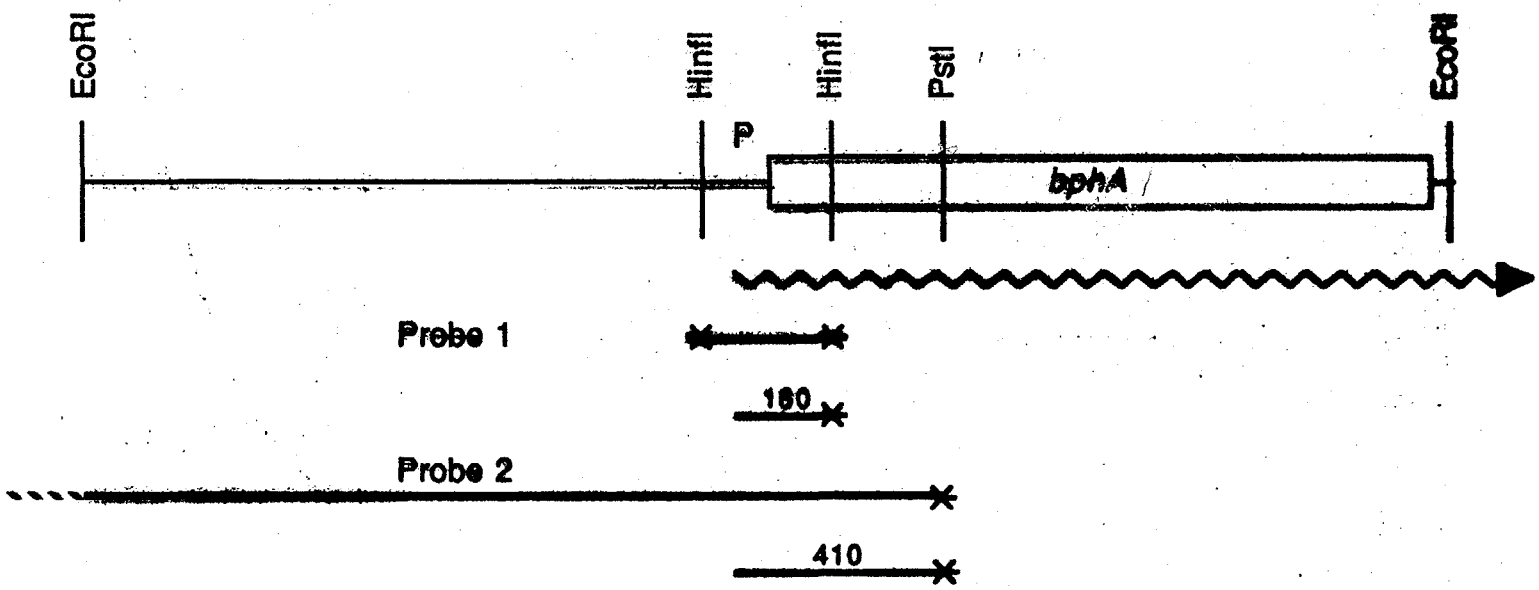
[a]Indicated as the percent of degradation compared with biphenyl-grown LB400
Resting cell assay: Mix 1B, 2B
FM4100: HB101 (pGEM410)
FM4560: TB1 (pGEM456)
LB400 (S): grown using succinate

Key: % Degradation

● 80-100
● 60-79
● 40-59
• 20-39
o 1-19

Sequence of 50% bph M





Both probe sequences localise promoter (P) to same region

Evidence that bph gene cluster is mobile:

✓ 1) Striking similarity between LB400 and H850 PCB degrading genes

- conserved 15 kb region
- otherwise no similarity

✓ 2) Two copies of bph gene cluster in JB1

- different flanking regions
- Different regions of chromosome
- different control ?

✓ 3) Spontaneous bph⁻ mutants

- frequency 1/40 (typically $1/10^7$)
- specific deletions
- entire bph cluster gone

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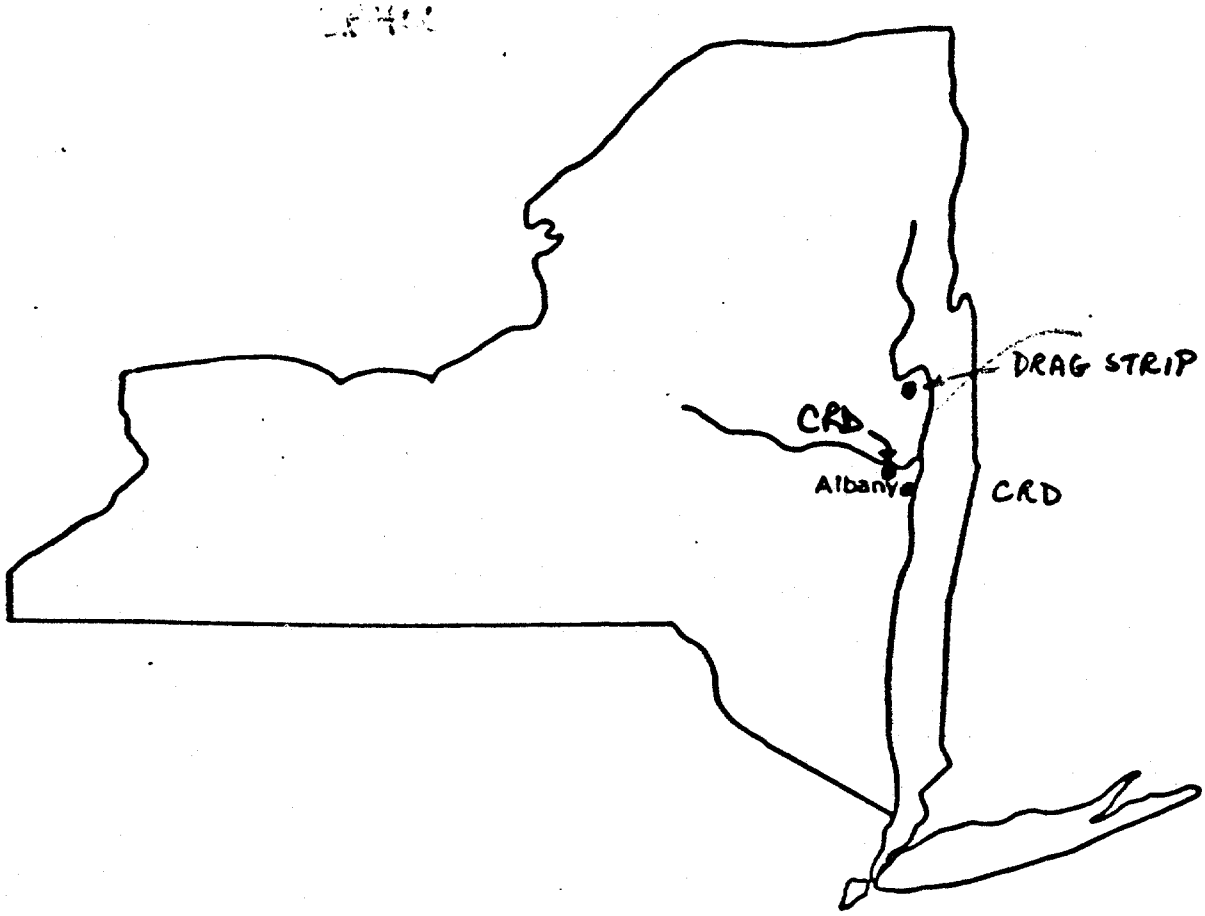
South Glen Falls Dragstrip

Field Test



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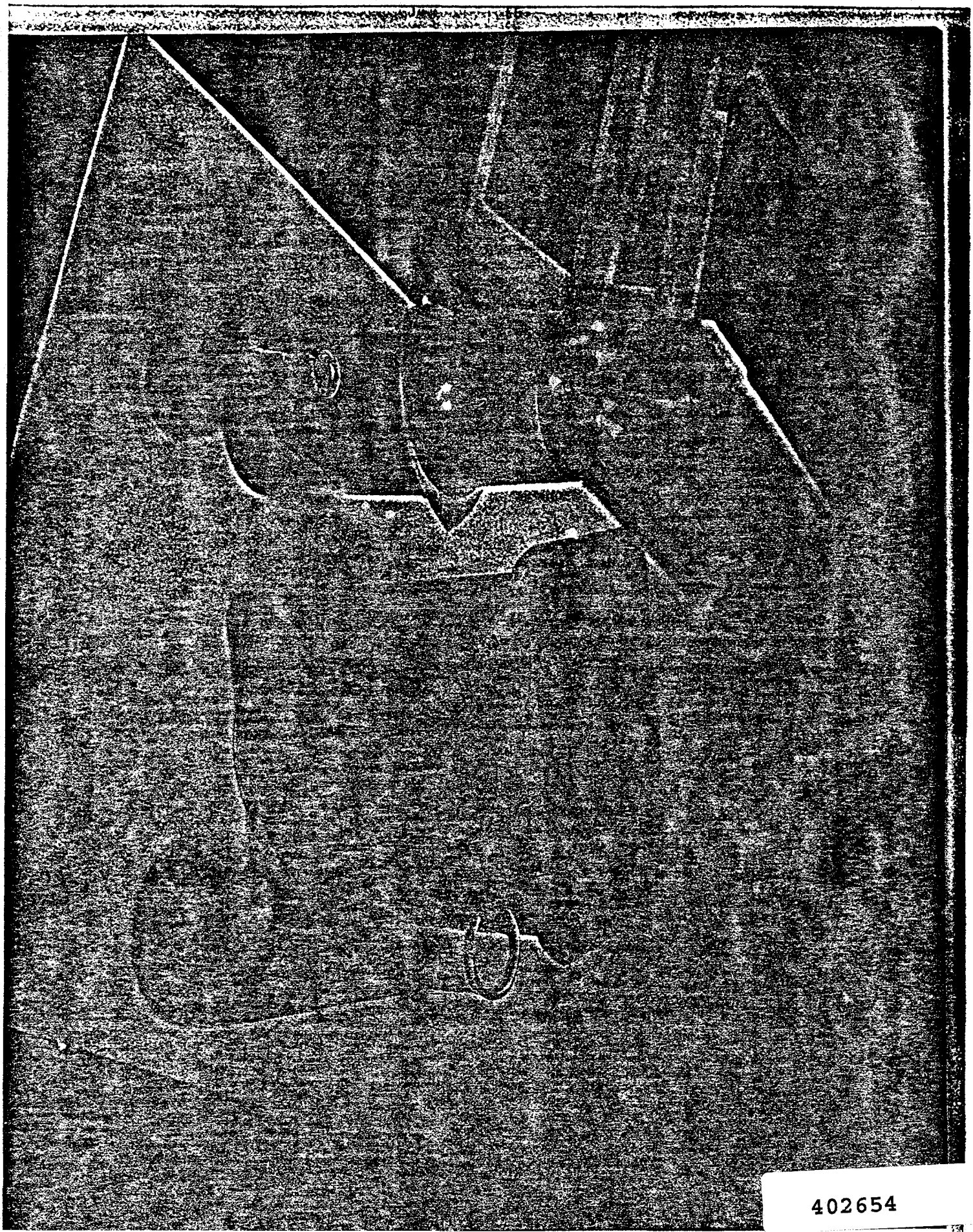

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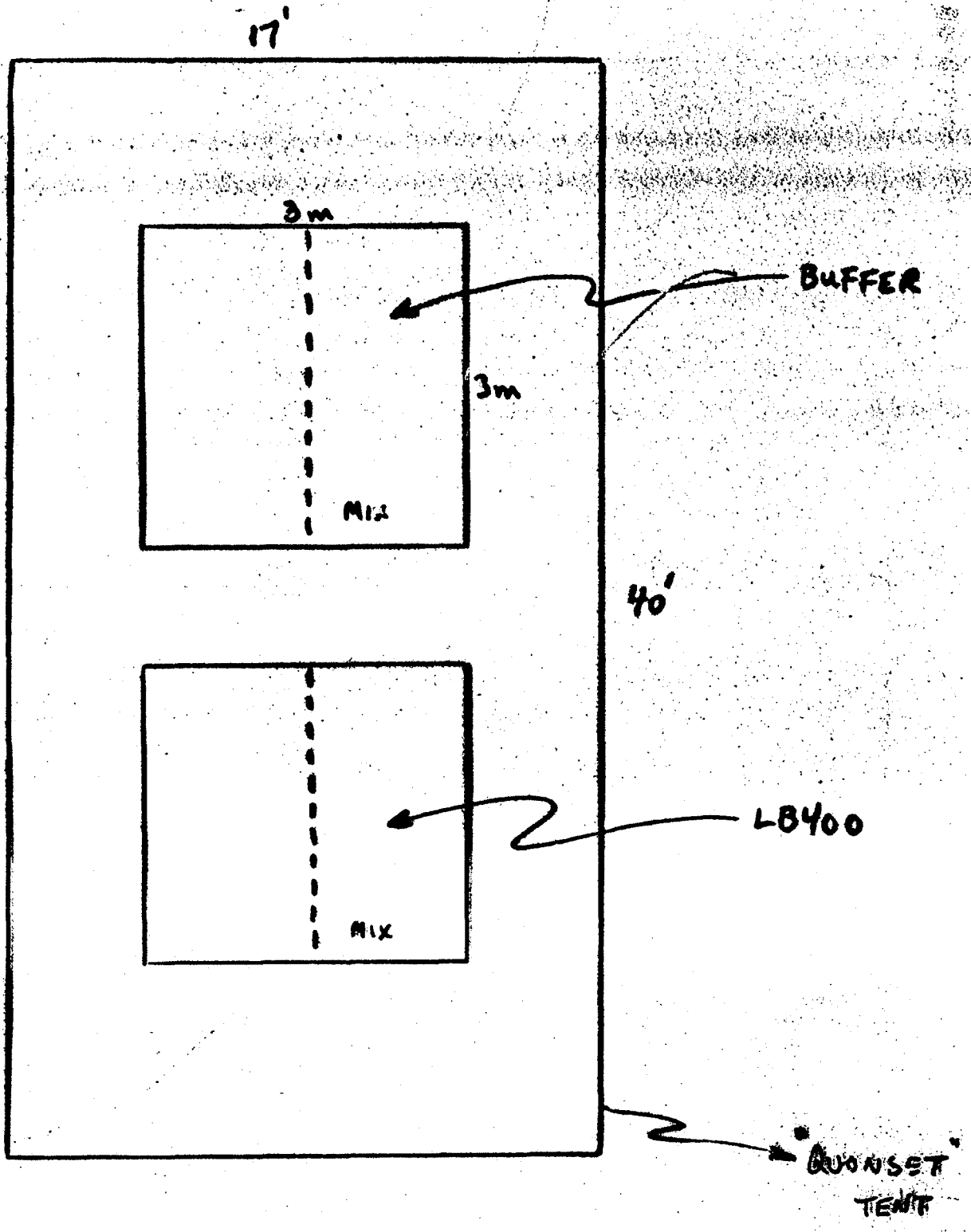
DRAG STRIP SITE TEST

SUMMER 1987



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Drag Strip Field Trial:

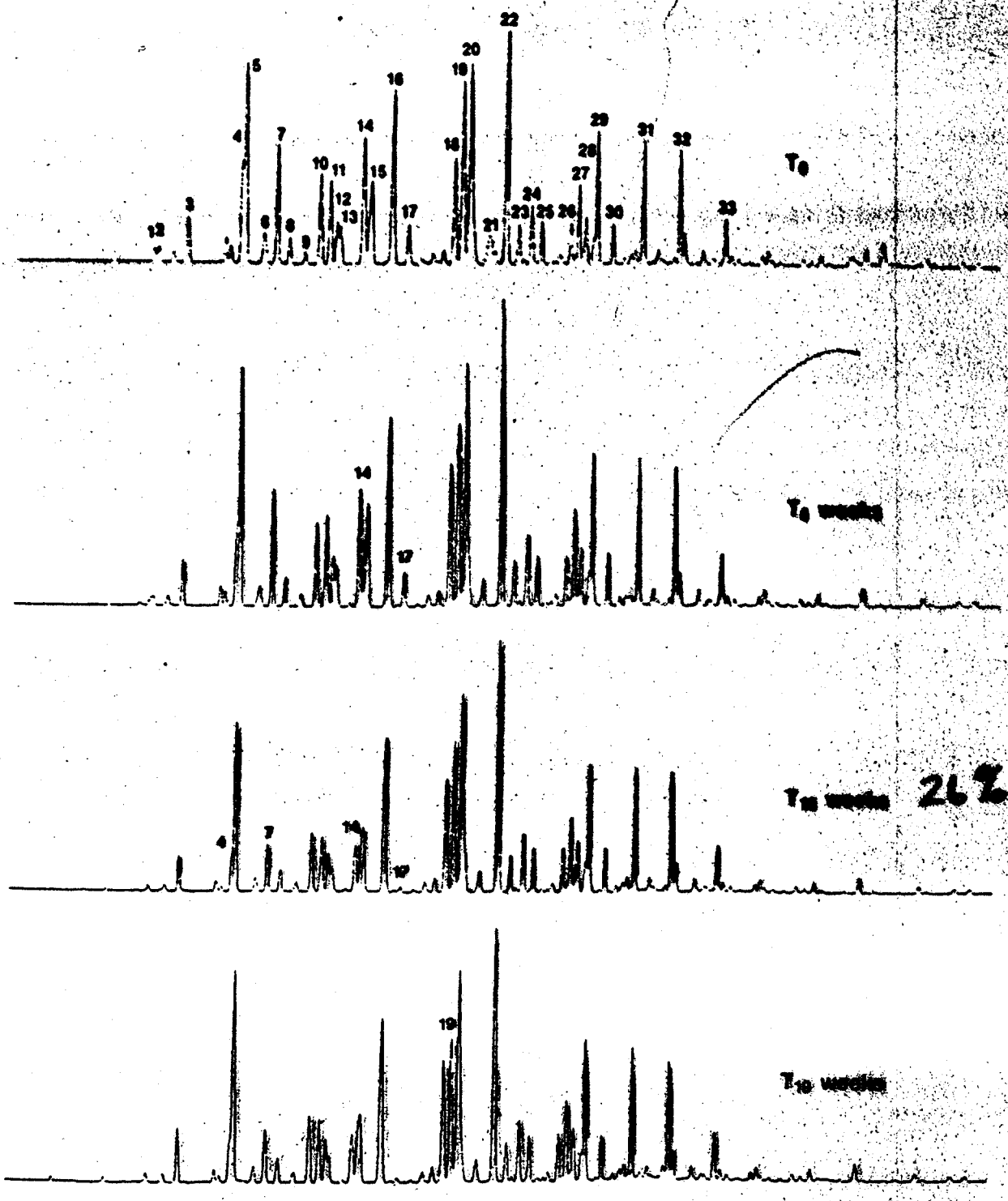


Figure 1-1. Time course of PCB biodegradation at drag strip field trial. Soil samples were taken weekly, extracted, and analyzed (GC) for PCB composition. Samples shown here were from the top centimeter of the unmixed, experimental plot (LB400 dosed).

Stirred plot 19% (1-15 cm) 20,000,000 PCBs/bacterium

control clean

Biodegradation Results:

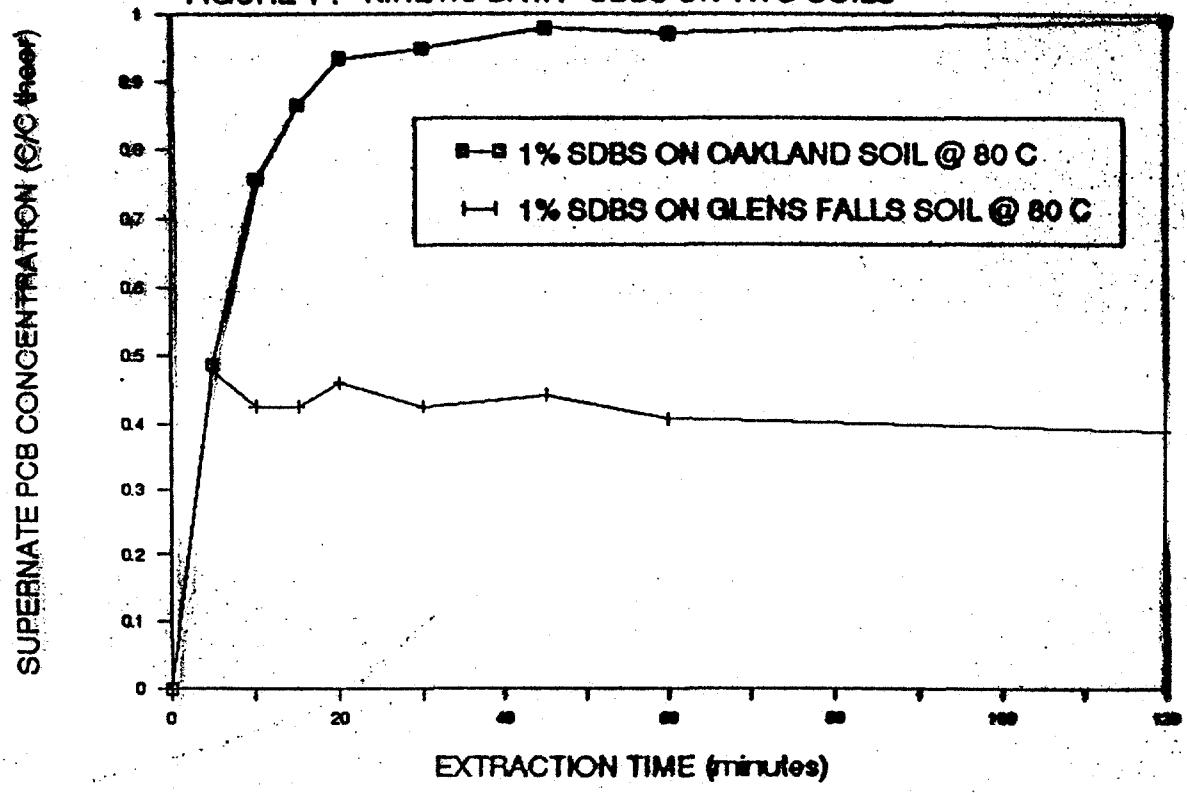
10-20% soil
10 OD JB1P3C
T= 3d (30°C)

Aroclor 1242 >90%

Dragstrip '89
Soxhlet Extract ≈70%
On soil ≈35%

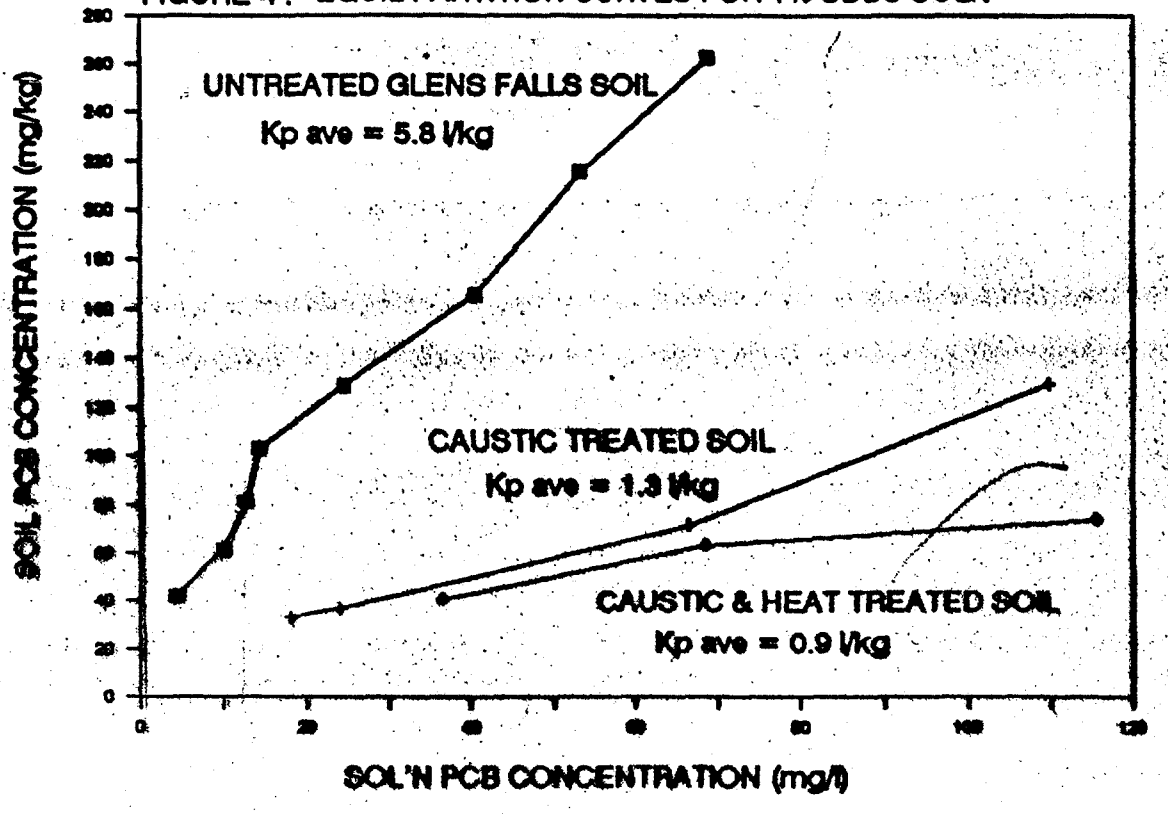
PCB EXTRACTION FROM GLENS FALLS SOIL

FIGURE 1 : KINETIC DATA - SDBS ON TWO SOILS



PCB EXTRACTION FROM GLENS FALLS SOIL

FIGURE 4 : EQUIL PARTITION CURVES FOR 1% SDBS SOLN



Biodegradation Results: 10-20% soil
10 ob JB1F3C
T = 3d (30°C)

Dragstrip '89 (100 ppm)

Untreated	35% degradation
Caustic treated	60% "
Caustic + heat	?

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Aroclor 1242

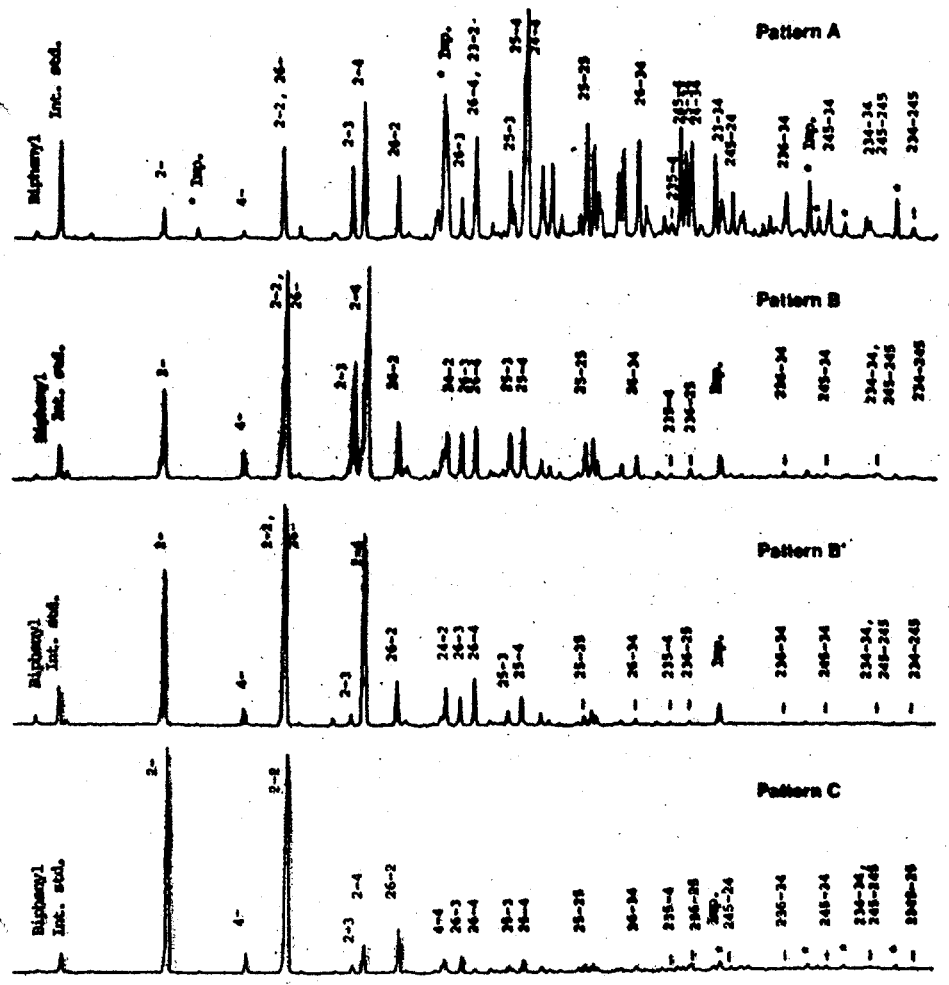


Fig. 1. DB-1 capillary gas chromatograms (plots of detector response versus elution time) of upper Hudson River sediments that show surface pattern A (largely unchanged Aroclor 1242) and subsurface patterns B, B', and C. A flame ionization detector was used so that the PCB peak response was nearly proportional to molar concentration; however, non-PCB impurities in the samples also produced observable peaks (designated * and Imp.). The major PCB congeners responsible for the observed peaks are designated by the numbers that correspond to the position of chlorines on each of the two phenyl rings; thus 2-2 and 24-4 indicate 2,2'-dichlorobiphenyl and 2,4,4'-trichlorobiphenyl, respectively. Internal standard peaks are designated Int. std.

B-B' and C were associated with the deeper "hot spots," which have been estimated to contain 77 metric tons of PCBs (*6*).

Quantitation of the individual capillary GC peaks indicated that the levels of most tri- and tetrachlorobiphenyls were depressed relative to those in Aroclor 1242 in all classes of upper Hudson River sediments, but particularly in those that showed patterns B, B', or C. Summary data for 2,5,4'- plus 2,4,4'-chlorobiphenyl (CB) and for 2,5,3',4'-CB (which are representative of congeners with lesser or greater responsiveness to dechlorination, respectively) are shown in Table 1. Conversely, in all sediment classes the levels of the 2,6,2'- and 2,6,3'-CBs and those of all dichlorobiphenyls were increased two- to sixfold, and the levels of the monochlorobiphenyl 2-CB increased 7- to 70-fold, with the largest changes observed in the samples that showed patterns B, B', or C (Table 1). The increases in the mono- and dichlorobiphenyls occurred despite their greater tendency to elute into the river water or undergo aerobic biodegradation. Thus it was evident that in the upper Hudson River as a whole a massive (40 to 70 metric tons) conversion of tri-, tetra-, and higher chlorobiphenyls to mono-, di-, and 2,6,X'-trichlorobiphenyls (X' = 2, 3, or 4) had occurred, particularly in the subsurface (15- to 30-year-old) portion of the sediments.

The sediments of Silver Lake, a 10-ha urban pond in Pittsfield, Massachusetts, contain an estimated 29 metric tons of PCBs (*10*), which are believed to have originally been almost entirely Aroclor 1260 released from adjacent transformer-manufacturing operations before 1972. A mapping and

Aroclor 1260

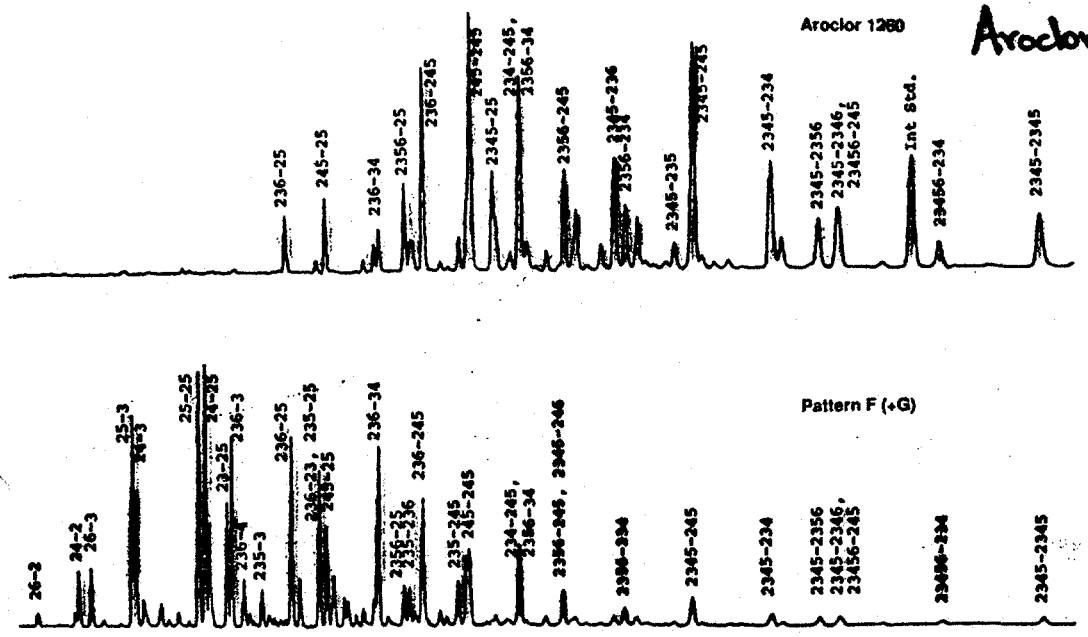


Fig. 2. DB-1 capillary gas chromatograms (plots of detector response versus elution time) of Aroclor 1260 and of the Aroclor 1260 residue extracted from a Silver Lake sediment composite that showed mainly pattern F (with some pattern G, which contributed the three small peaks on the left). These chromatograms were obtained with an electron capture detector. Such detectors give a stronger response with the more heavily chlorinated PCB congeners, a weaker response with the less heavily chlorinated ones, and little or no response with unchlorinated impurities. The major PCB congener peaks and the internal standard are designated as in Fig. 1.

402660

(JFB)

pattern of Cl-loss in contaminated sediments: selective

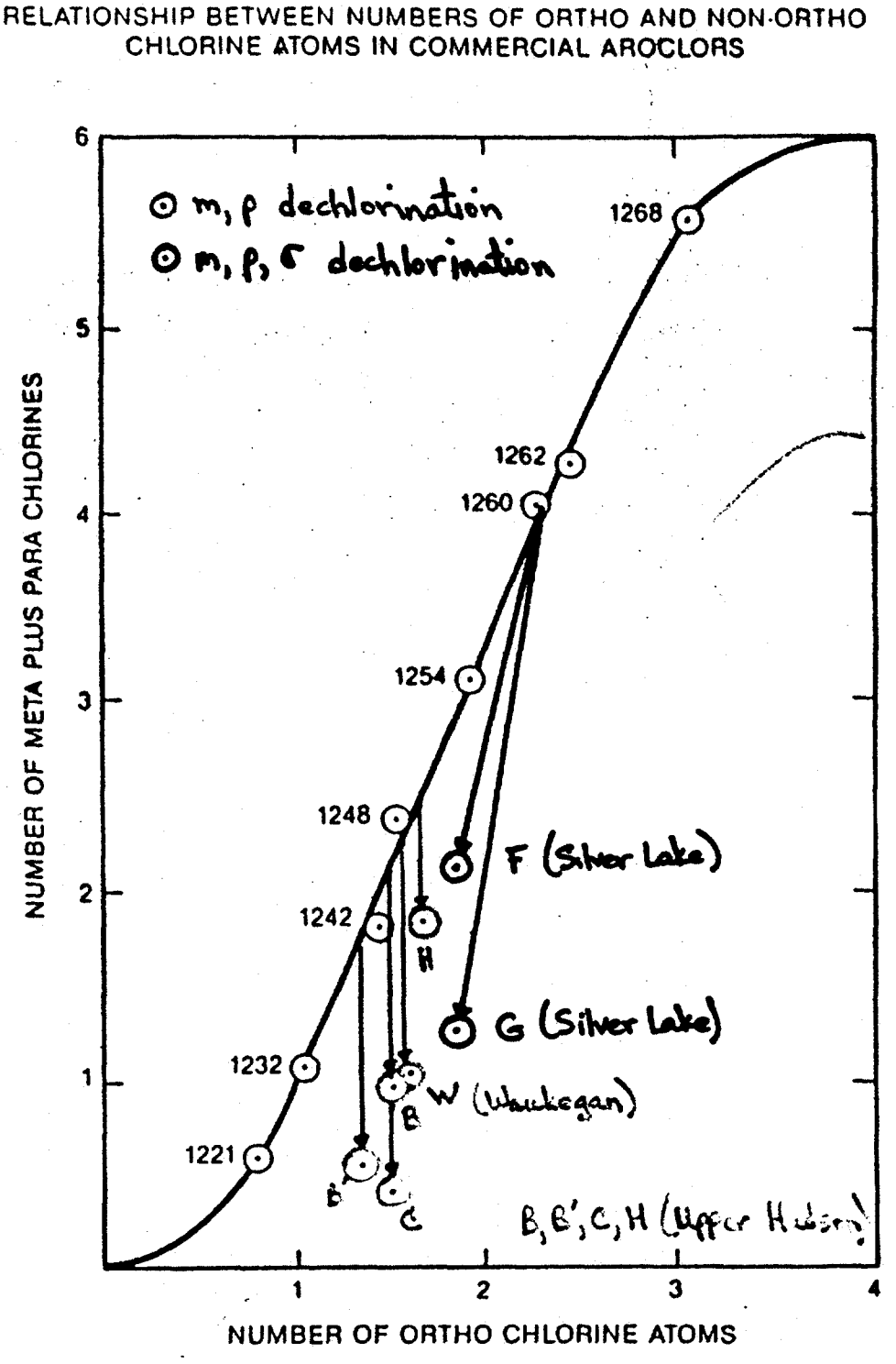
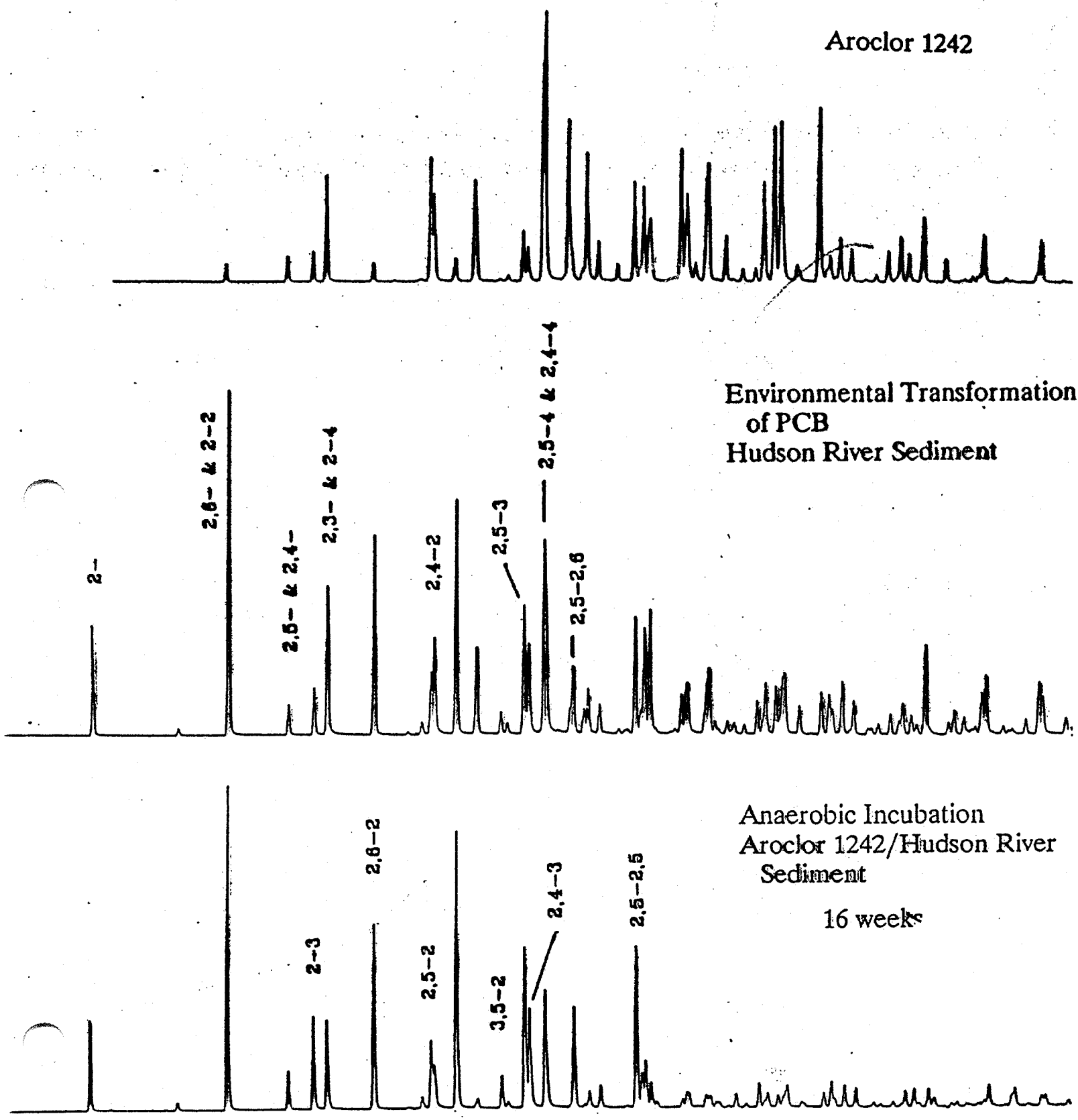


Figure 8-1. **Relationship between numbers of *ortho* and non-*ortho* chlorine atoms in commercial Aroclors.**

Comparison of Environmentally Transformed PCB and Laboratory Incubation of PCB



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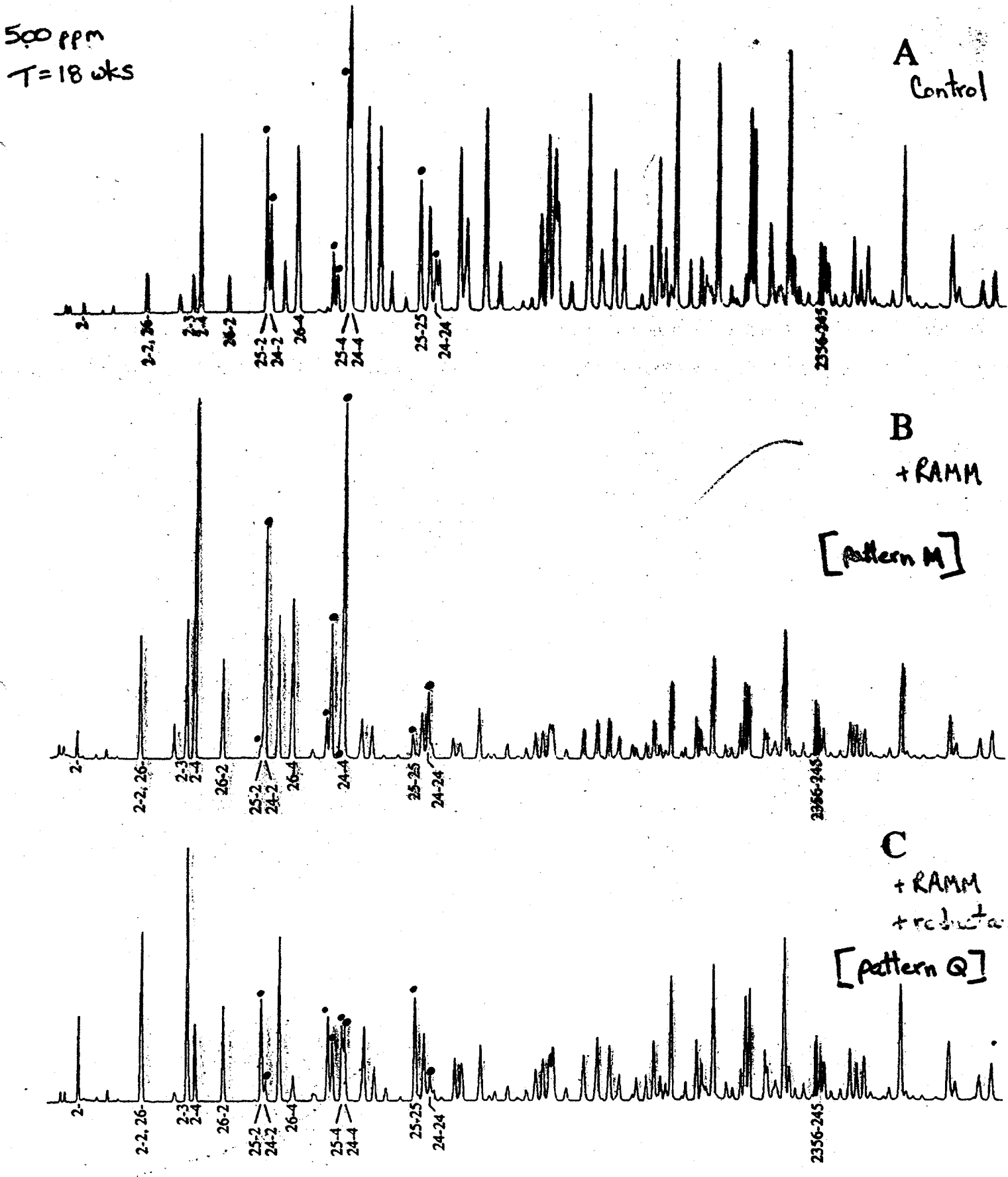
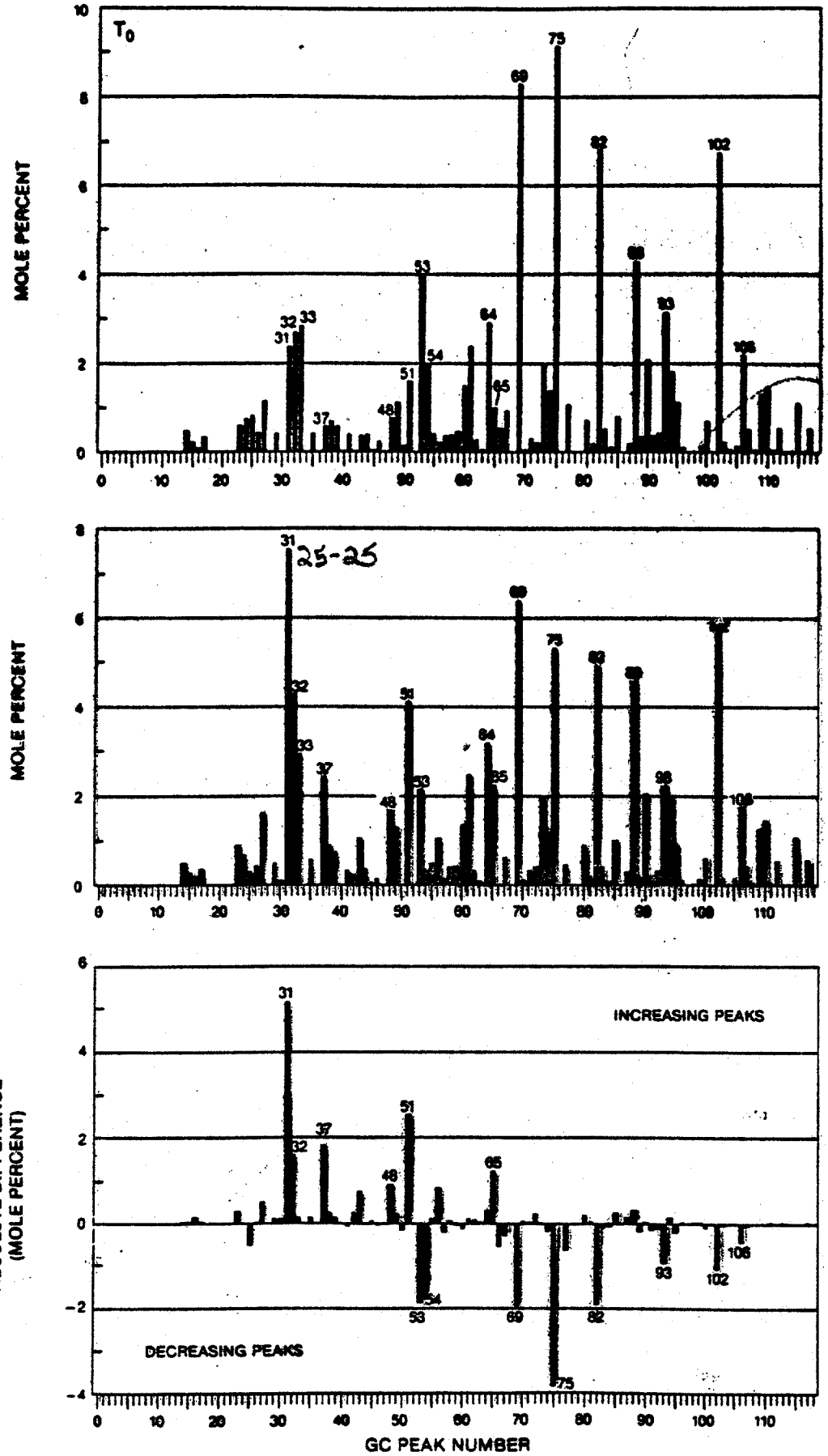


Figure 6-2: Dechlorination patterns observed under different conditions after 18 weeks. Panel A, autoclaved control; Panel B, includes RAMM (pattern M); Panel C, includes RAMM + cysteine hydrochloride at 1 gm/L (pattern Q).

• 25 –

• 24 –

Pattern H Dechlorination (para)
Woods Pond



25-34 added: 25-34-; 25-3-08 edited out

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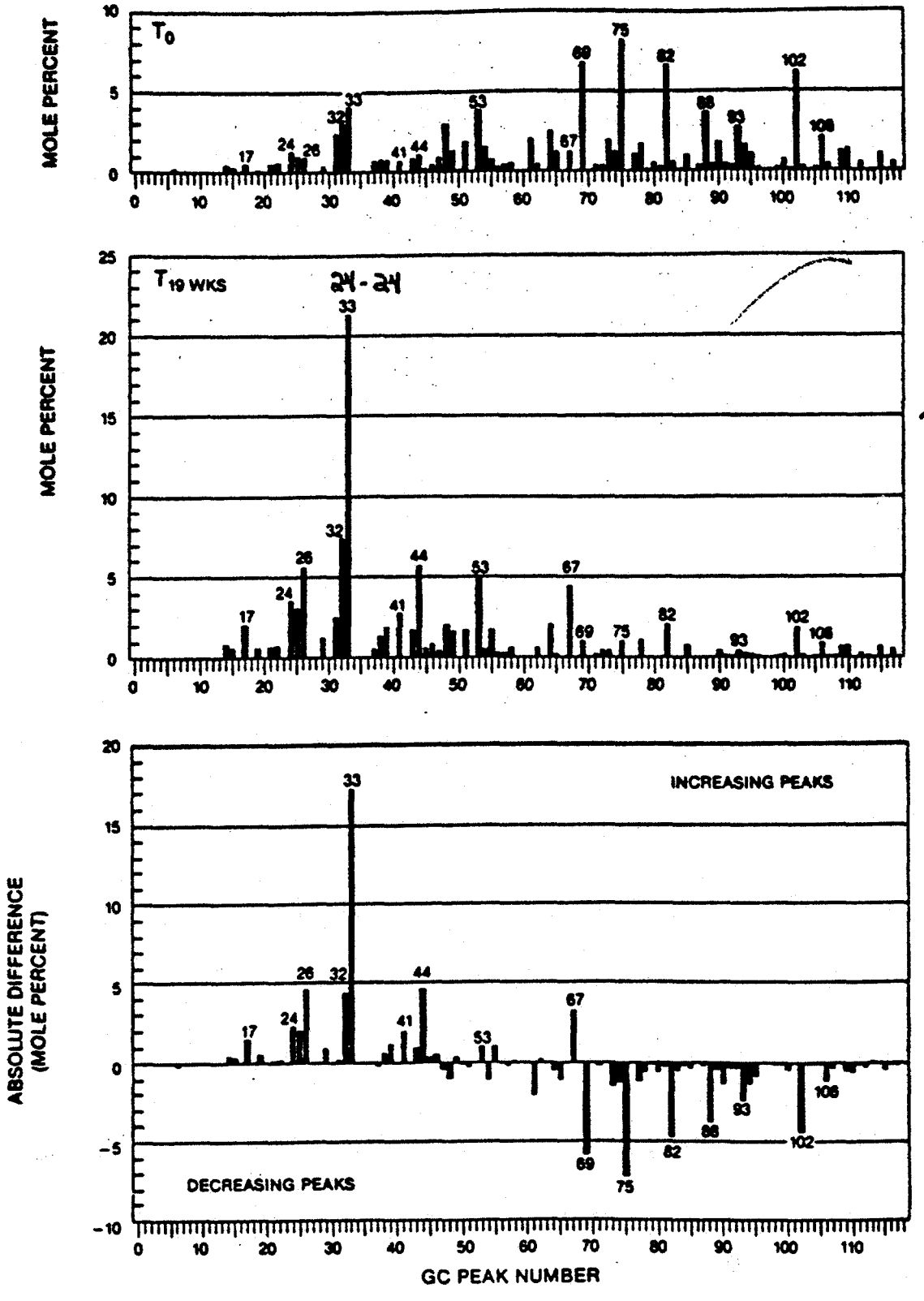
Pattern N Dechlorination (meta + para)
Woods Pond



T_0

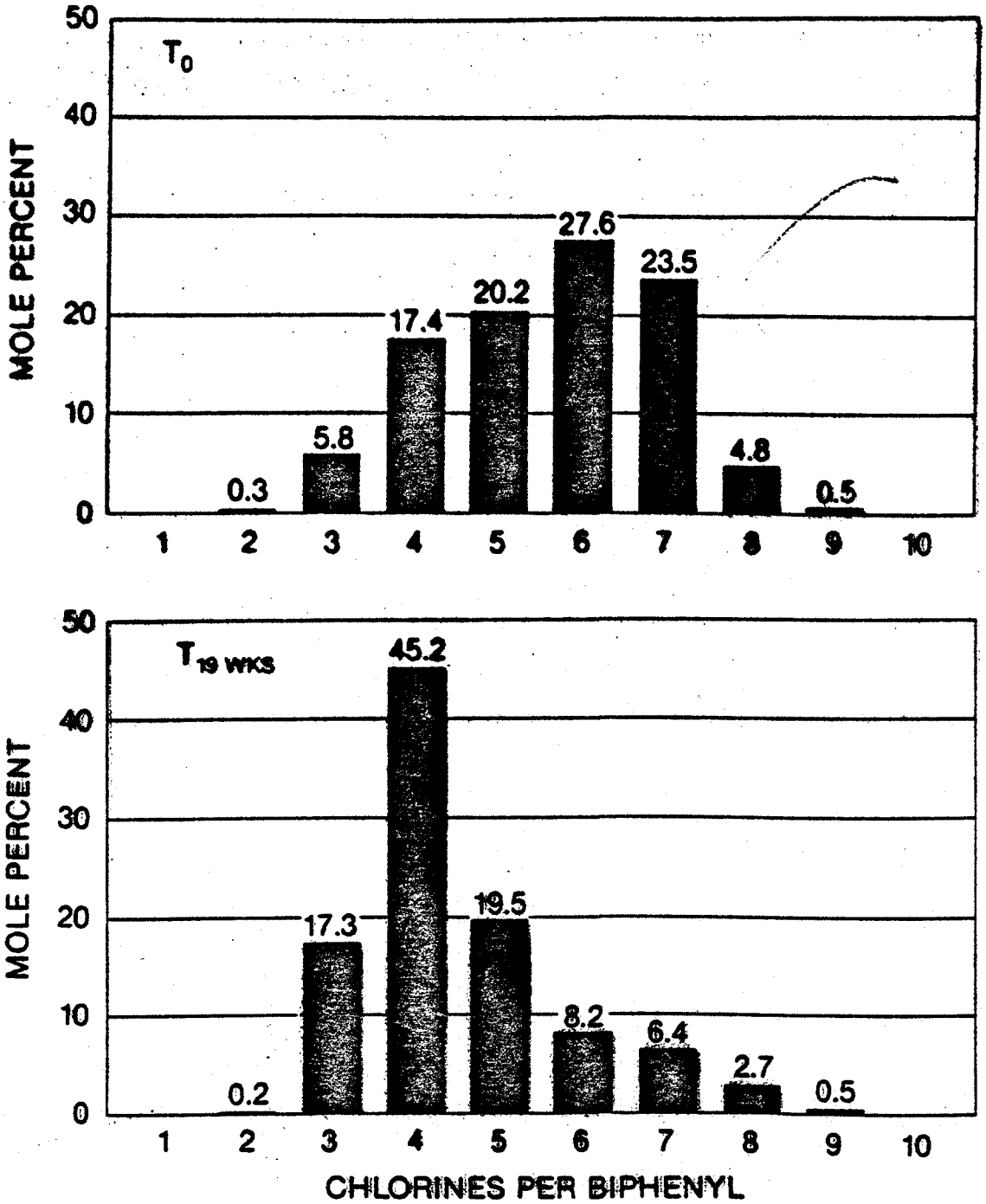
$T_{19\ wk}$

23456-CB added: 23456-, 2356-, 236-, 26-, 26-CB edited out

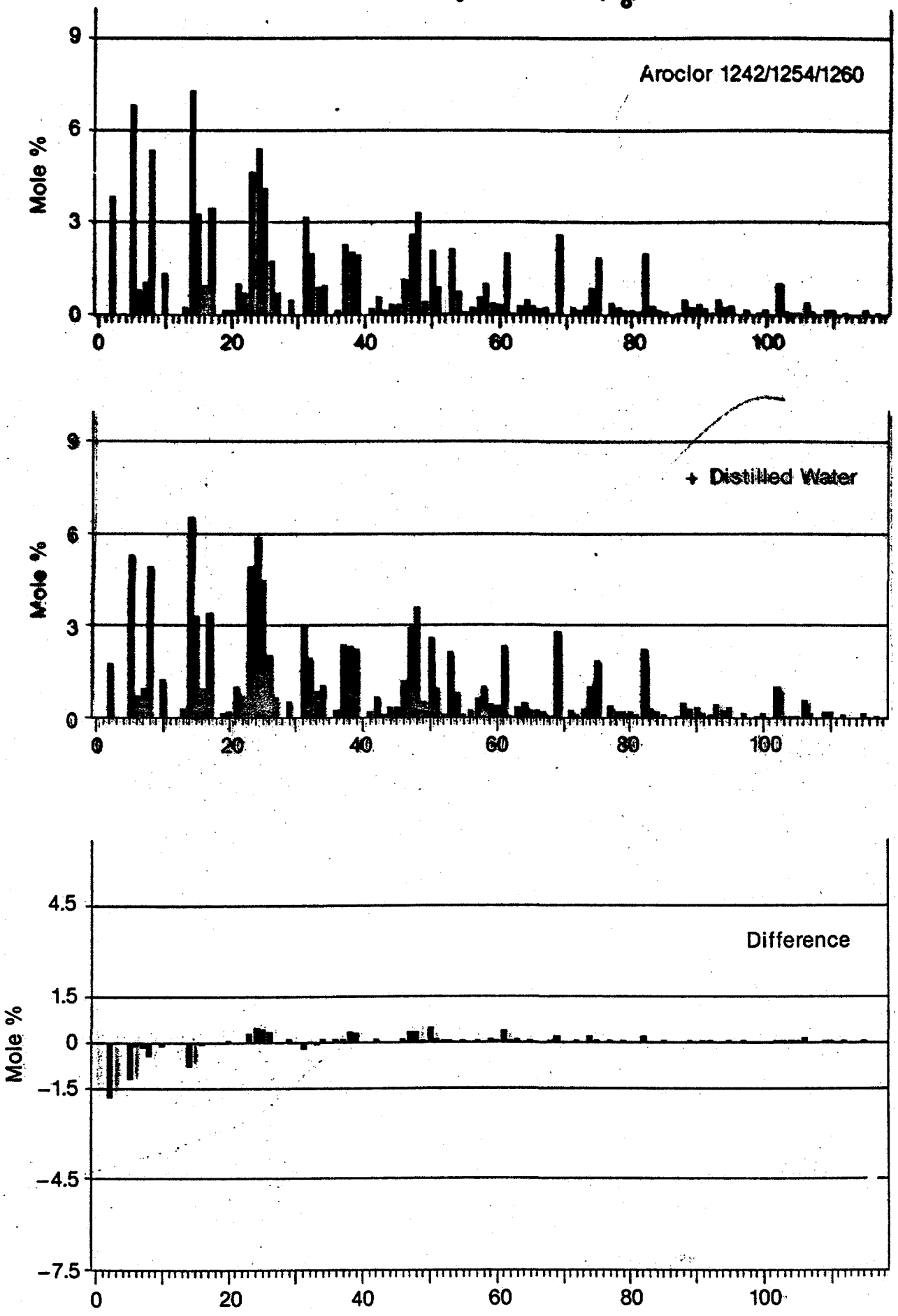
Pattern N Dechlorination

Woods Pond

hexa
hepta
octa
} reduced from 56 → 17 mole %

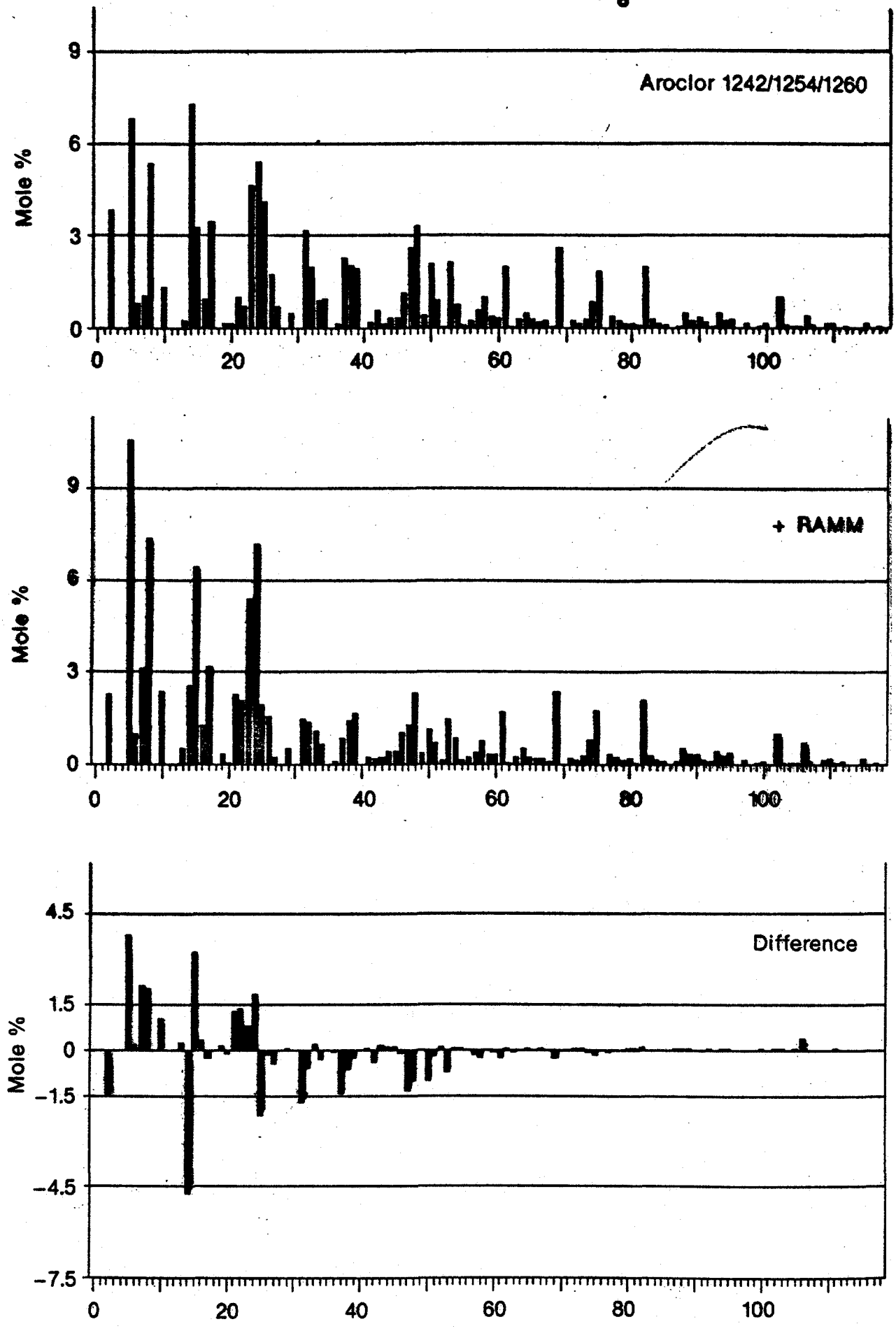


Water-only Addition (T_8)



402668

Nutrient Acceleration (T_8)



402669

Relative Dechlorination Rate	A	B	C	D
105%	+			
90	+	+		
105	+	+	+	
171	+	+	+	+
210	+	+ +	+	+
171	+ +	+ +	+	+
202	+	+		+
210	+	+	+ +	+
191	+	+		+ +

A = Phosphate Salts, Cystein, HCO_3^-

B = Nitrogen + Minerals ($CaCl_2$, $MgCl_2$, $FeCl_2$)

C = $MnCl_2$, MoO_4^{-2}, $CoCl_2$

D = Trace Metals (BO_3^{-3}, Zn^{+2}, Cu^{+2}, Ni^{+2}, SeO_3^{-2})

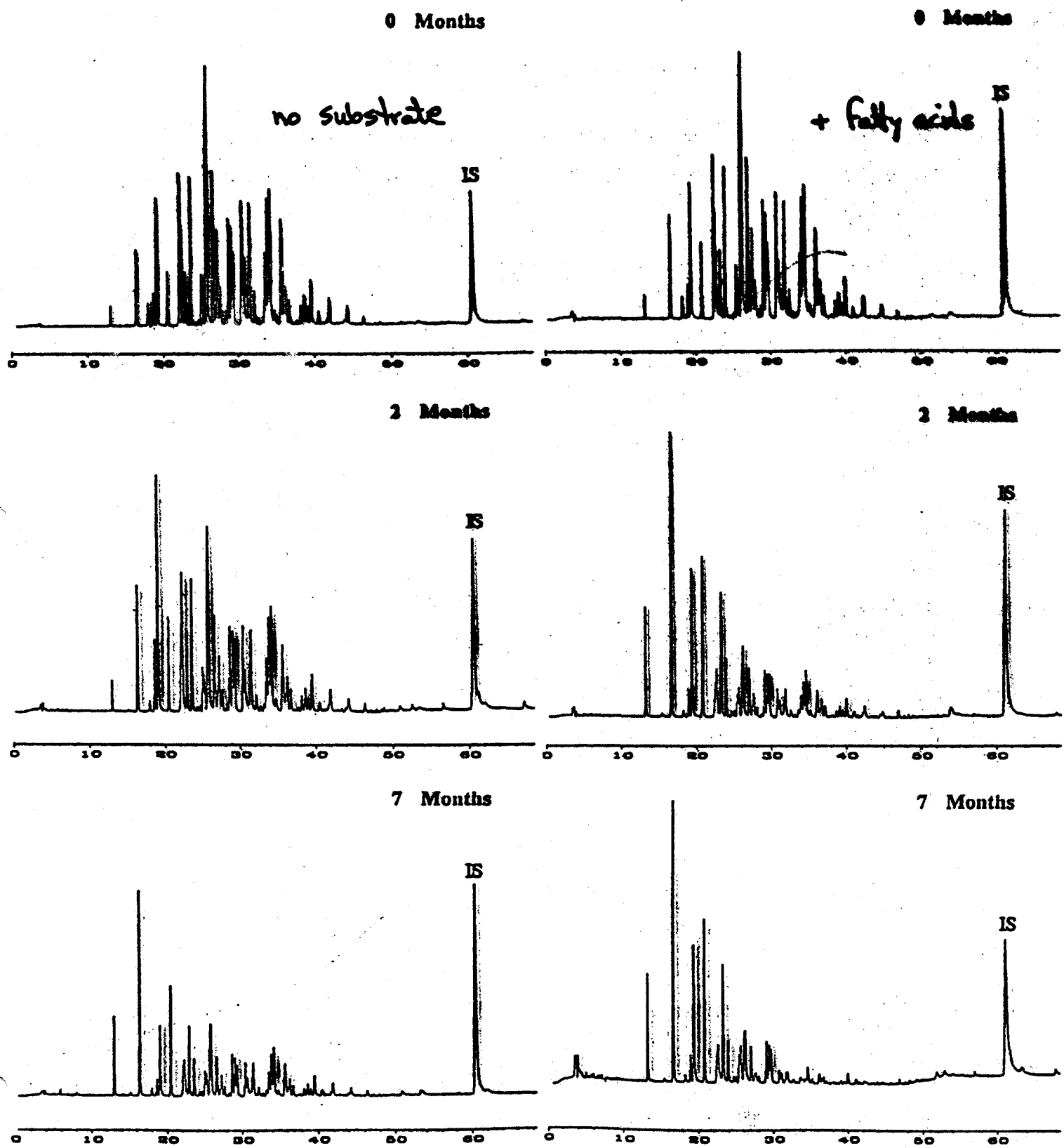
No Trace-Metals: $n = 3$, $\bar{x} = 25.7$, $v_n = 1.9$

Trace Metals: $n = 6$, $\bar{x} = 49.5$, $v_n = 4.2$

Increase (93%)

Effect of Carbon Addition (energy and/or reducing power):

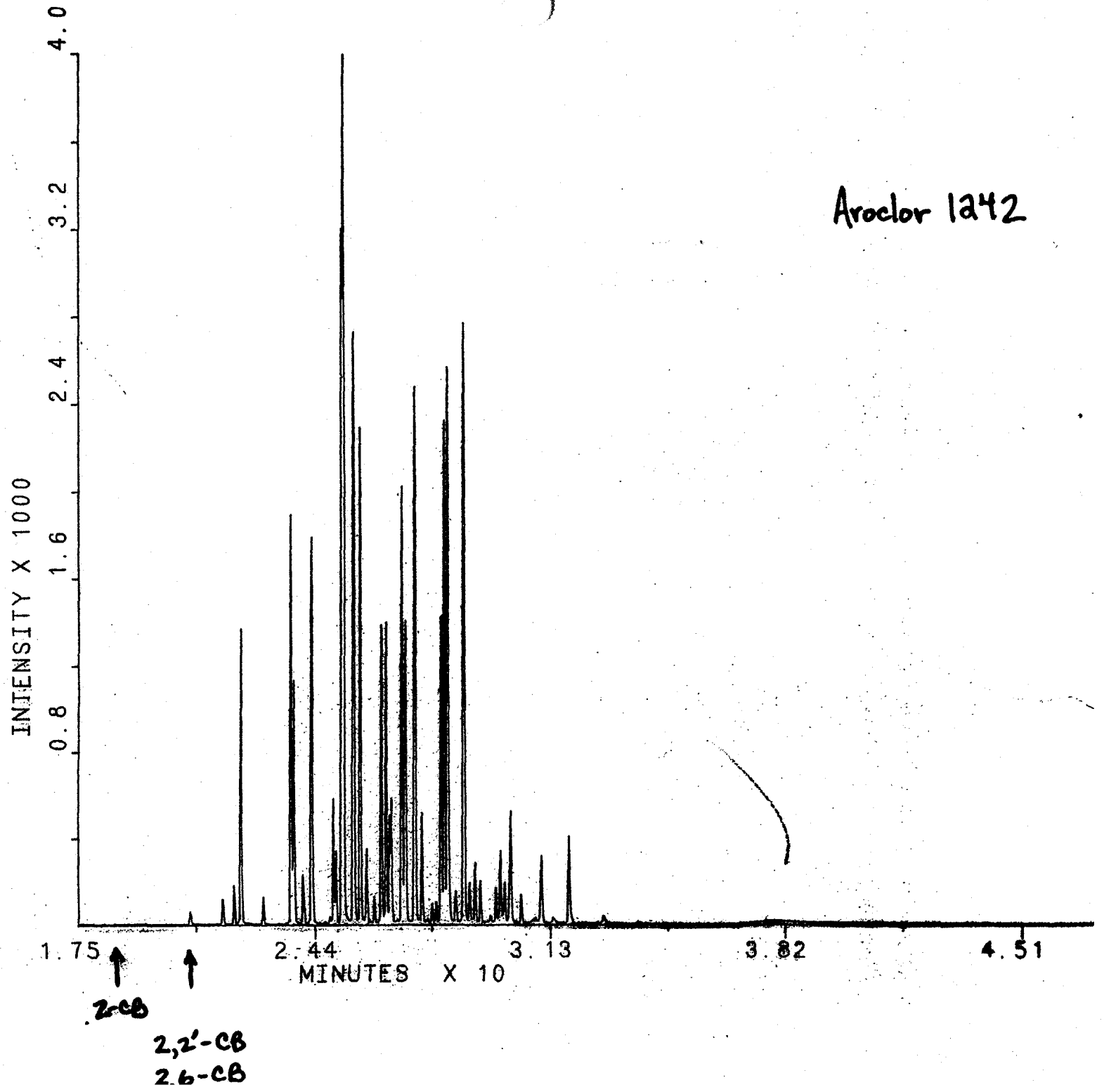
Young (NYU) fatty acids (acetate, propionate, butyrate, hexanoate)



Tiedje (MSU) pyruvate; Abramowicz (GE) YE, FTMBE

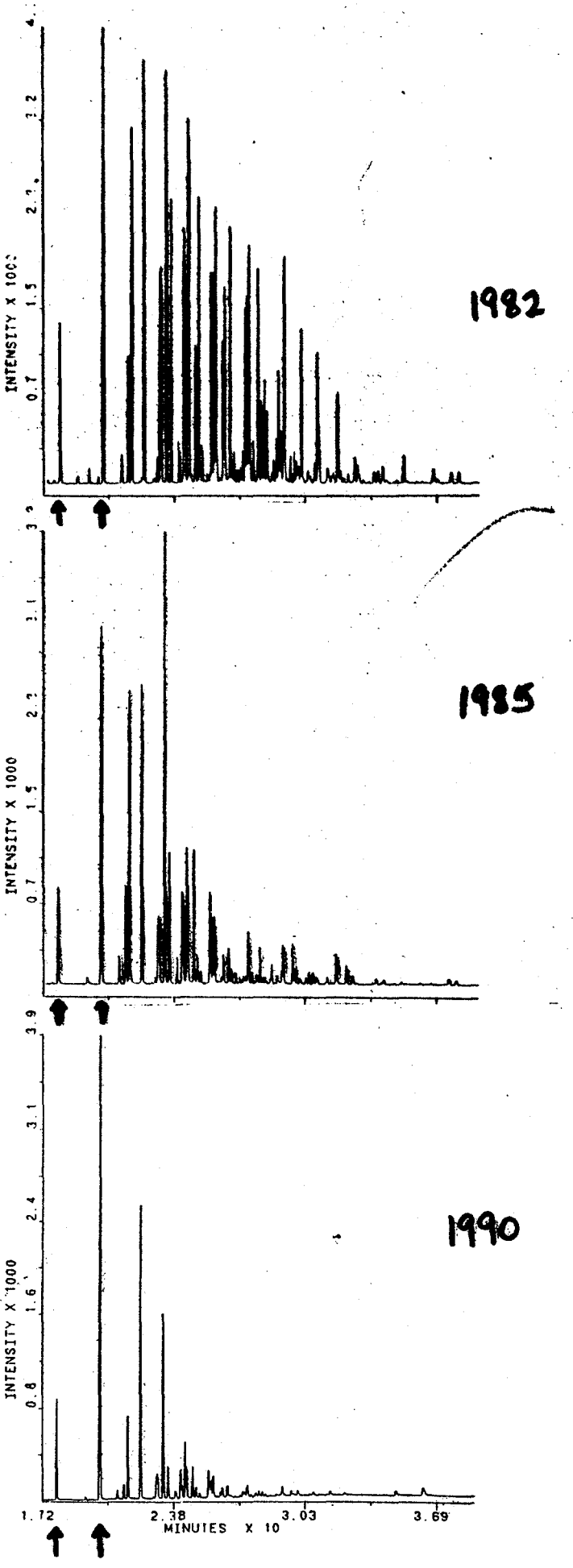
Vogel (U. Mich) methanol, glucose

402671

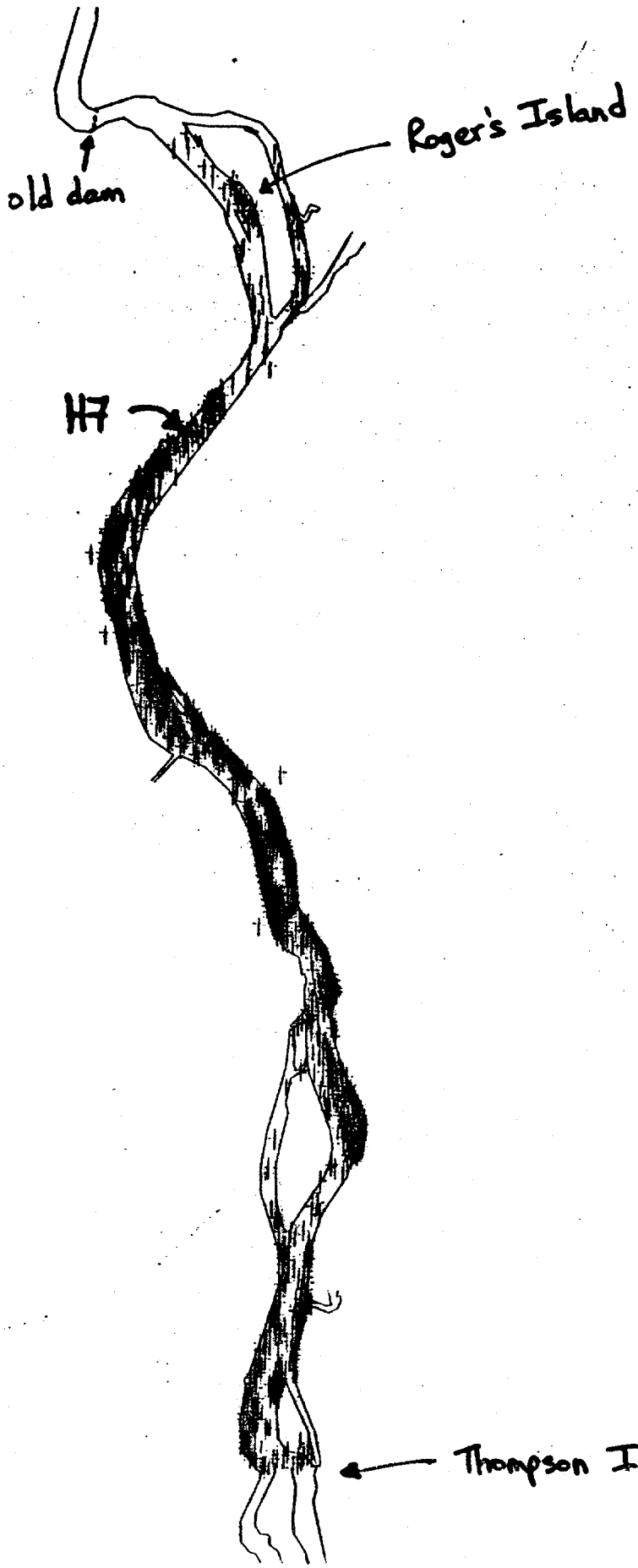

Aroclor 1242
INTENSITY X 1000
4.0
3.2
2.4
1.6
0.8
1.75
2.44
3.13
3.82
4.51
MINUTES X 10
2-CB
2,2'-CB
2,6-CB

Hudson River

H7 Sediments
(S. Ft. Edward)



402673



1984 DEC survey

2073 samples

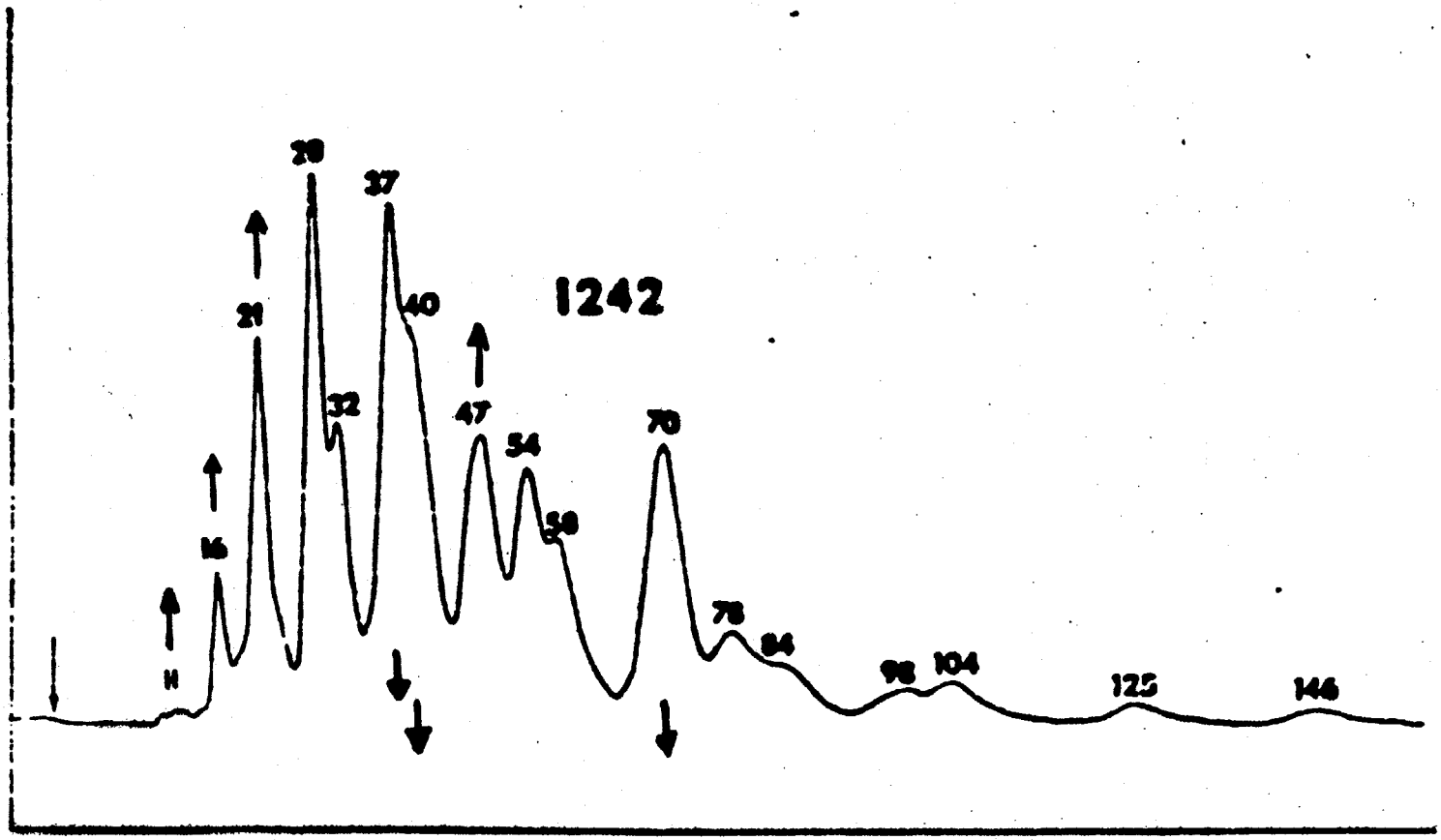
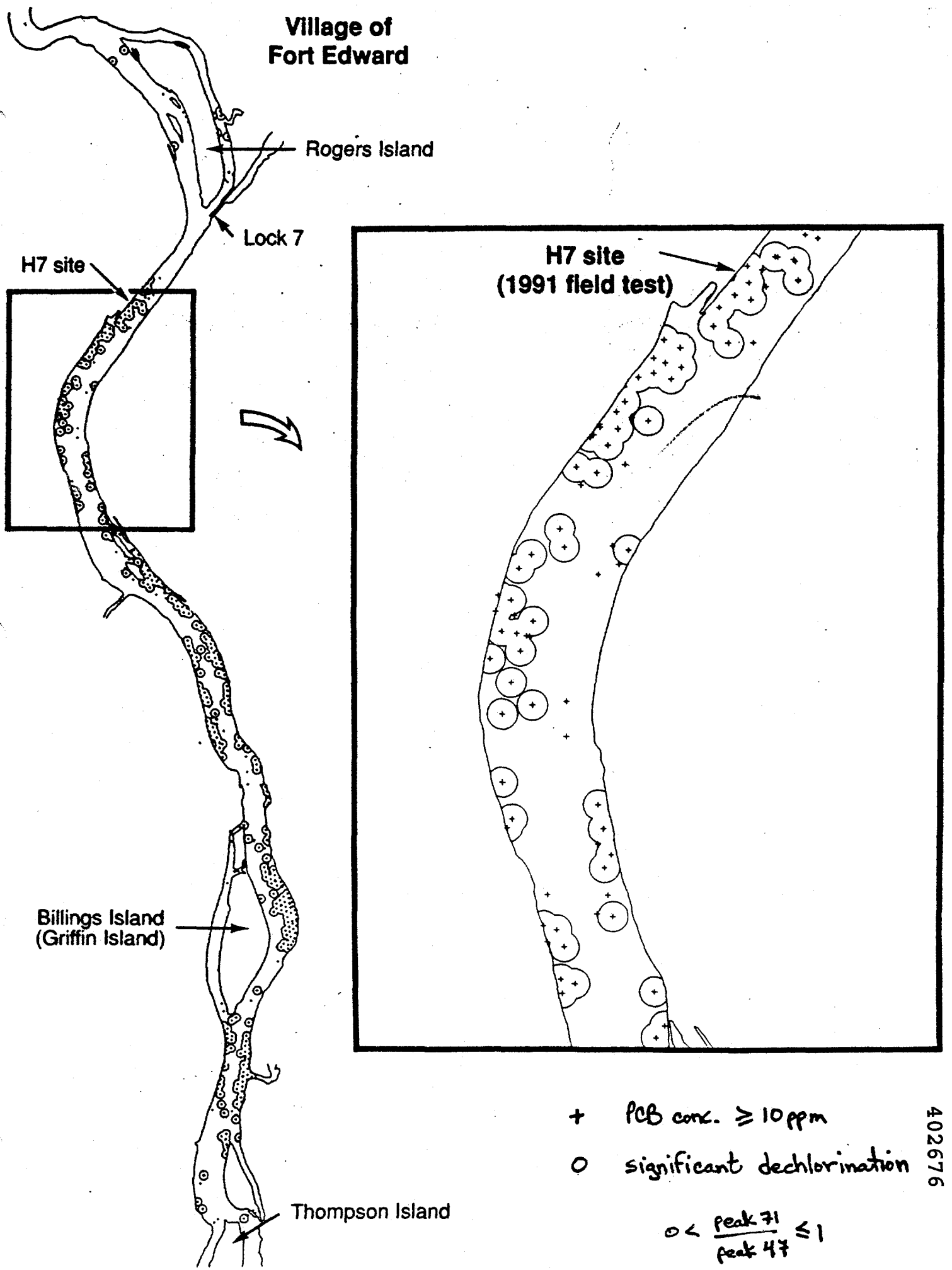


Figure 2. Gas chromatogram of Aroclor 1242 on SE-30 with an electrolytic conductivity detector. The peak identification numbers correspond to the retention time relative to p,p'-DDE=100. From injection, at the arrow, to peak 146 was about 20 min.


Village of
Fort Edward
Rogers Island
Lock 7
H7 site
H7 site
(1991 field test)
Billings Island
(Griffin Island)
Thompson Island
+ PCB conc. ≥ 10 ppm
O significant dechlorination
$0 < \frac{\text{peak 71}}{\text{peak 47}} \leq 1$

402676

Widespread Anaerobic Dechlorinating Microorganisms:

Contaminated Sediments

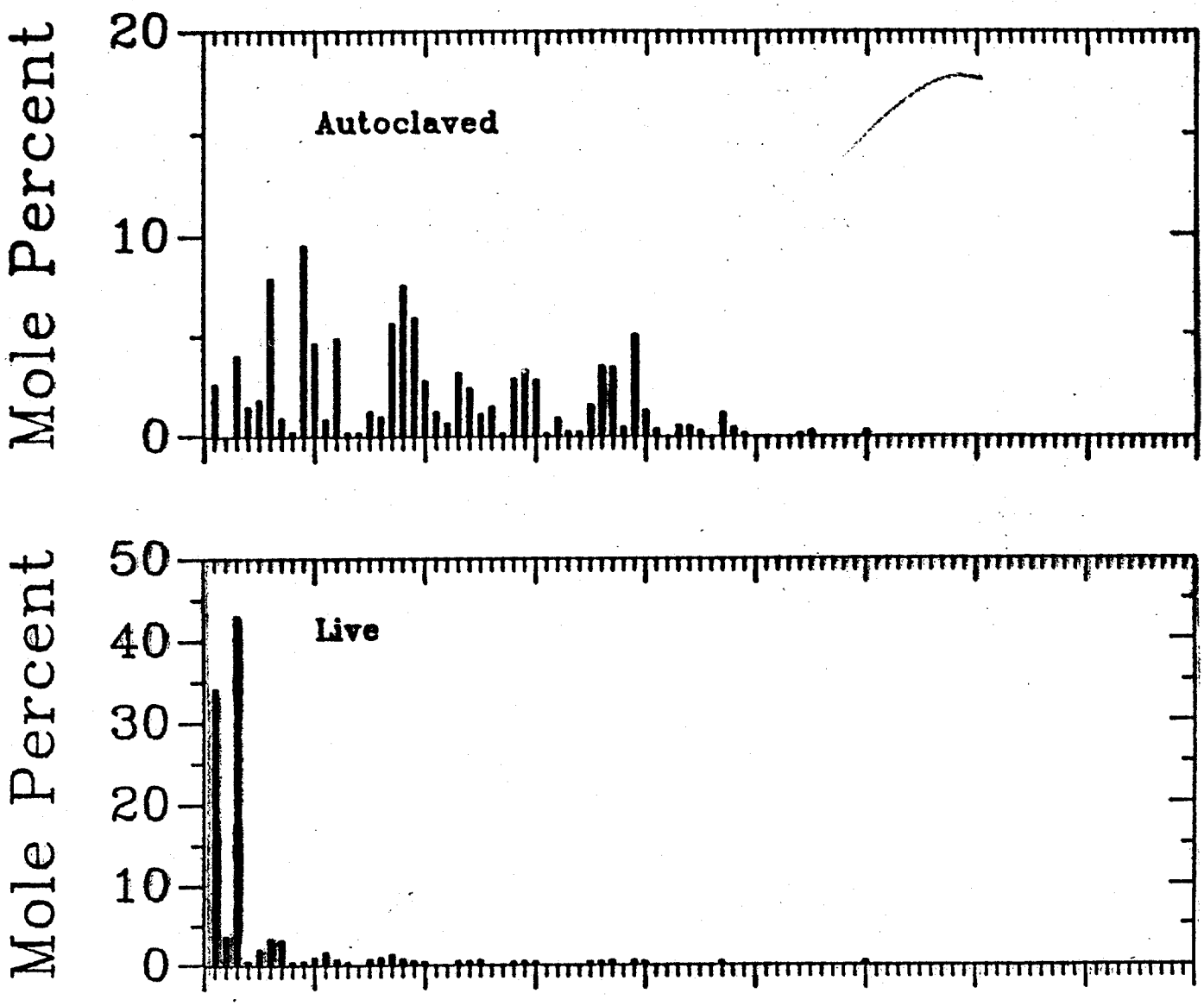
Escambia Bay, FL
Hoosic River, MA
Hudson River, NY
New Bedford Harbor, MA
Sheboygan River, WI
Silver Lake, MA
Waukegan Harbor, IL
Woods Pond, MA
Kalamazoo, MI

Uncontaminated Sediments

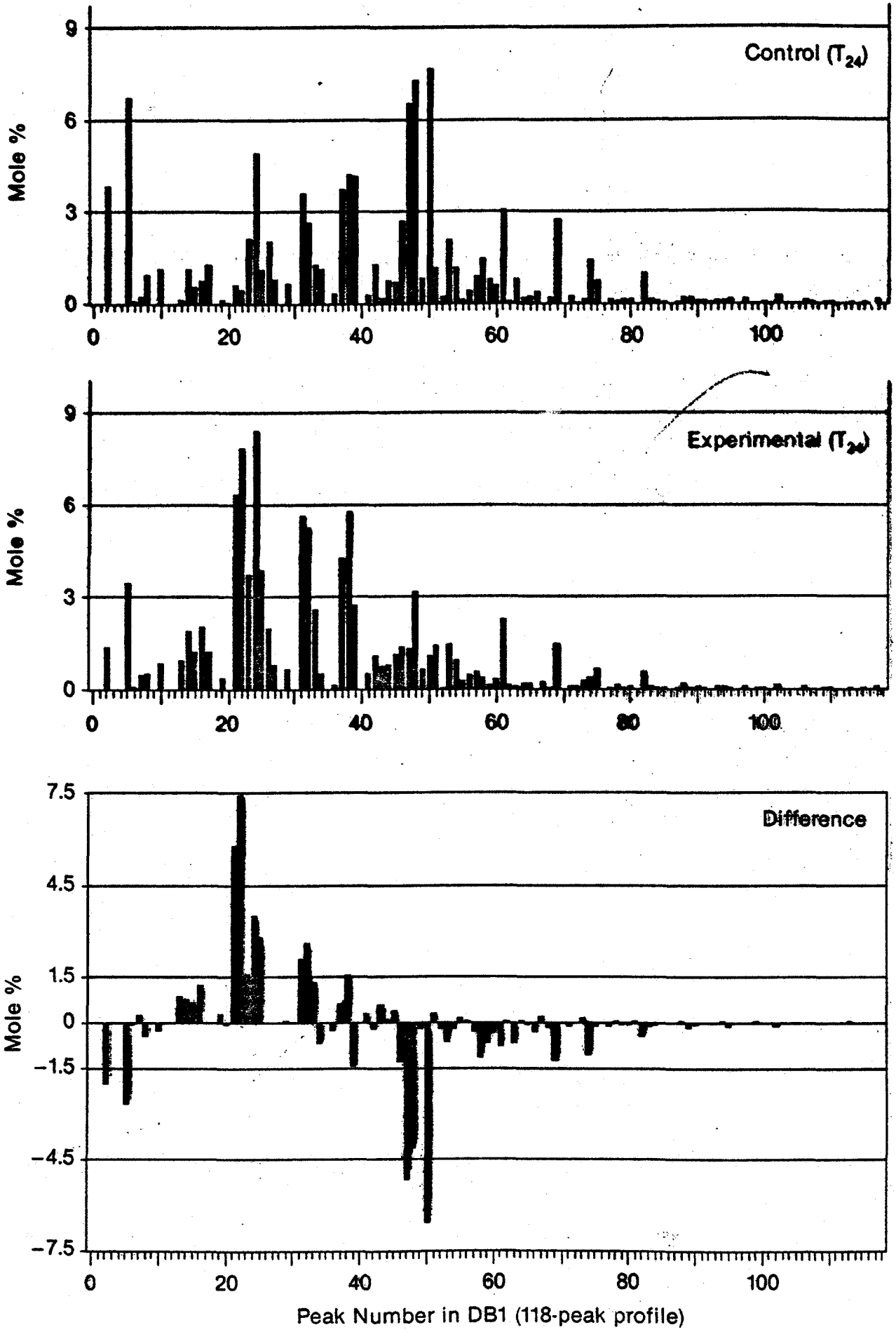
Adirondack Muck, NY
Center Pond, MA
Red Cedar River, MI
Saline River, MI
Spier Falls, NY (Hudson River)

Hudson River H7

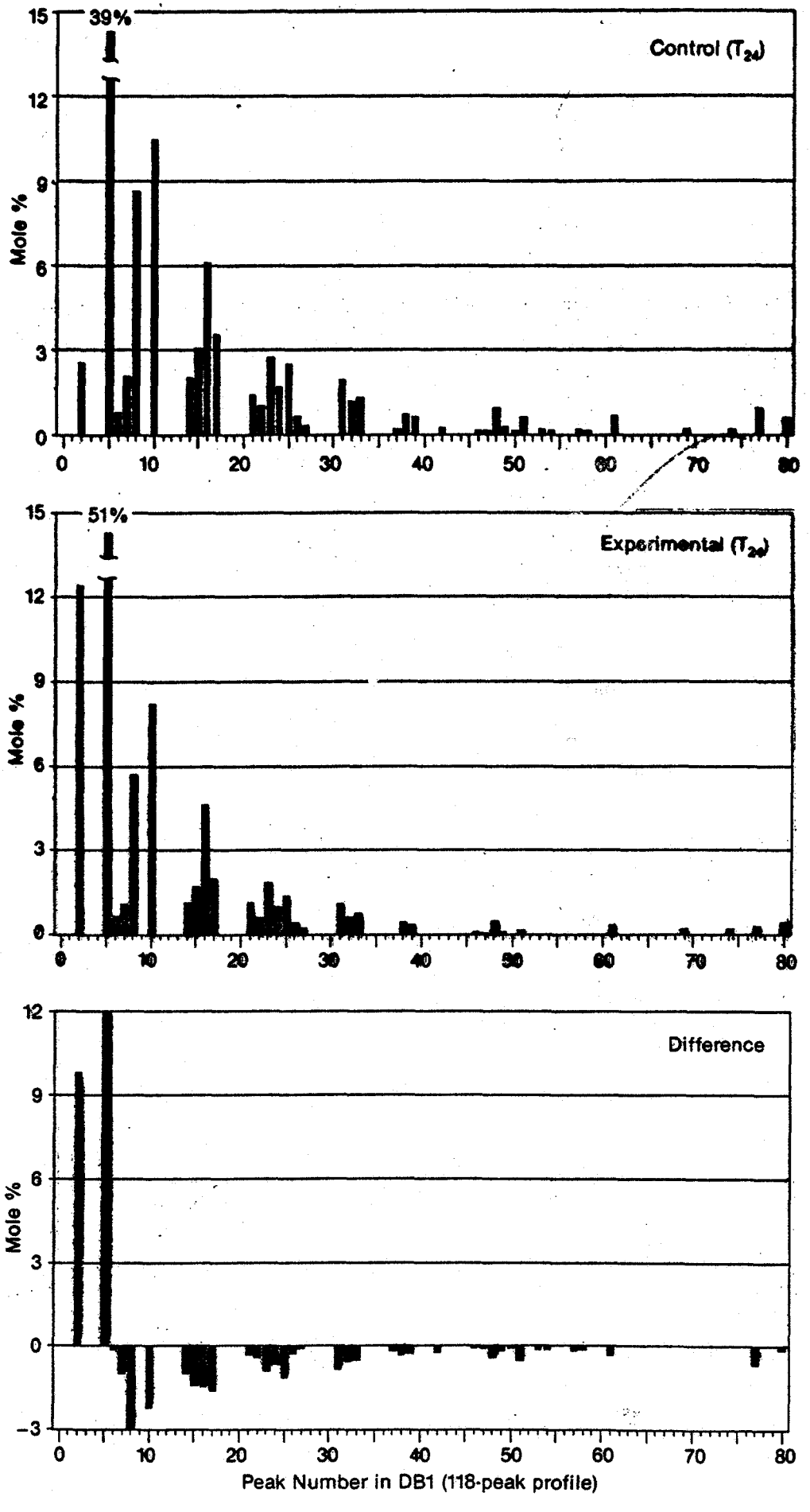
Aroclor 1242 16 Weeks



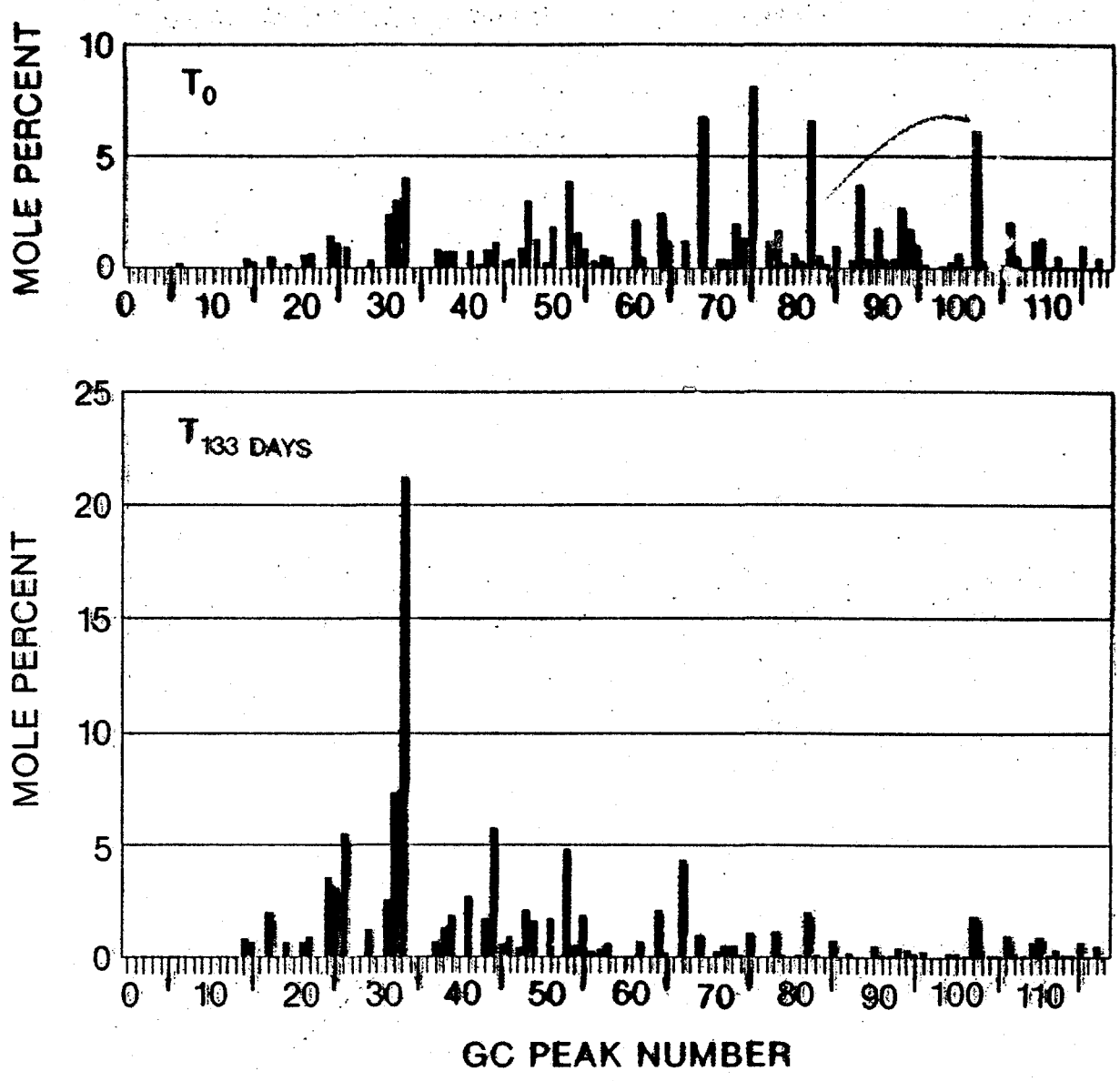
Endogenous Dragstrip PCBs



Endogenous Hudson River PCBs (H7)



PCB CONGENER DISTRIBUTION IN WOODS POND SEDIMENT BEFORE AND AFTER DECHLORINATION[1]



[1]SECOND TRANSFER, 23456-CB, 69-5A1-133

Aerobic Degradation

- Introduction
- rDNA Efforts
- Dragstrip

Anaerobic Dechlorination

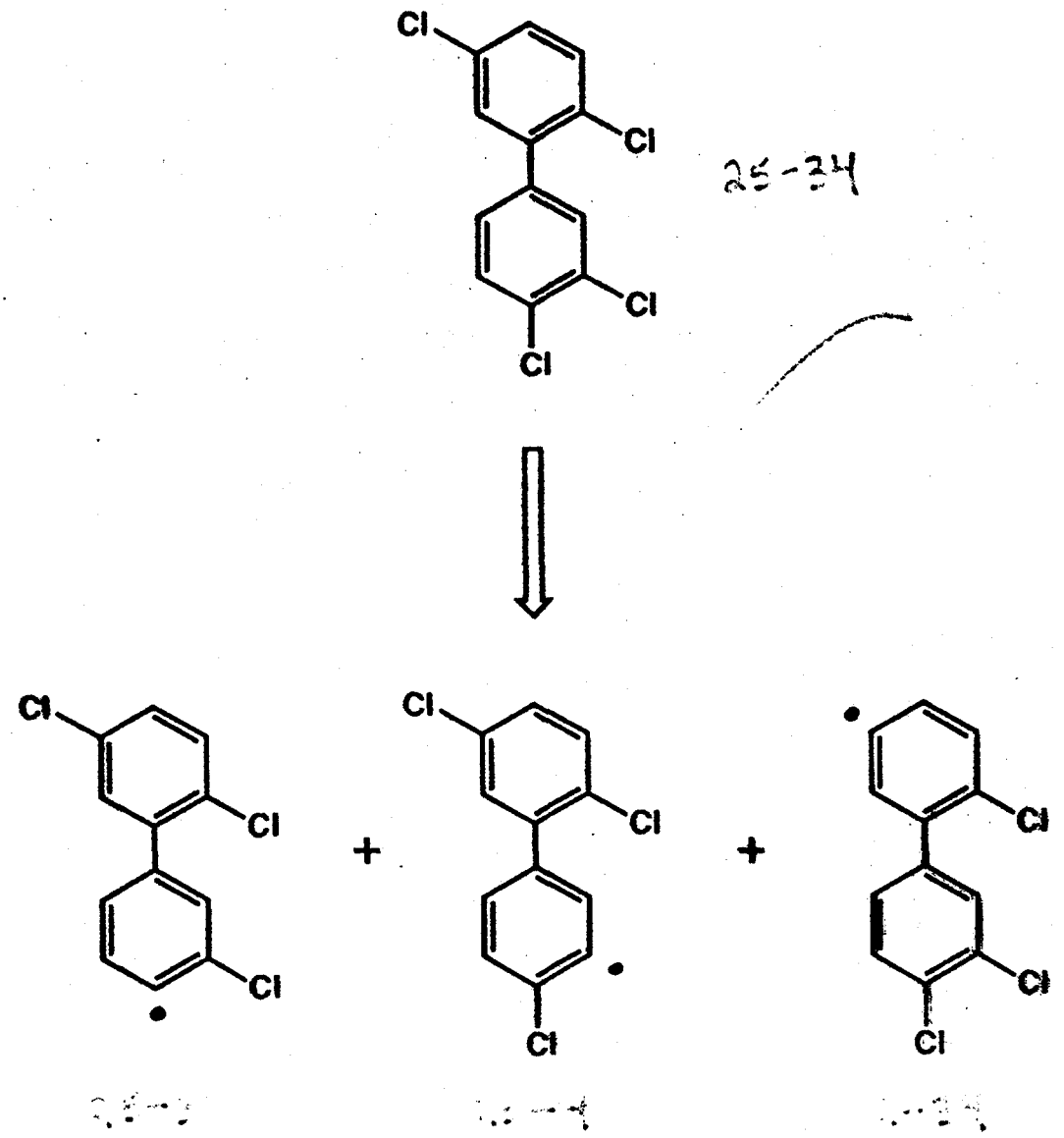
- Introduction
- Aroclors
- Single Congener

Anaerobic/Aerobic

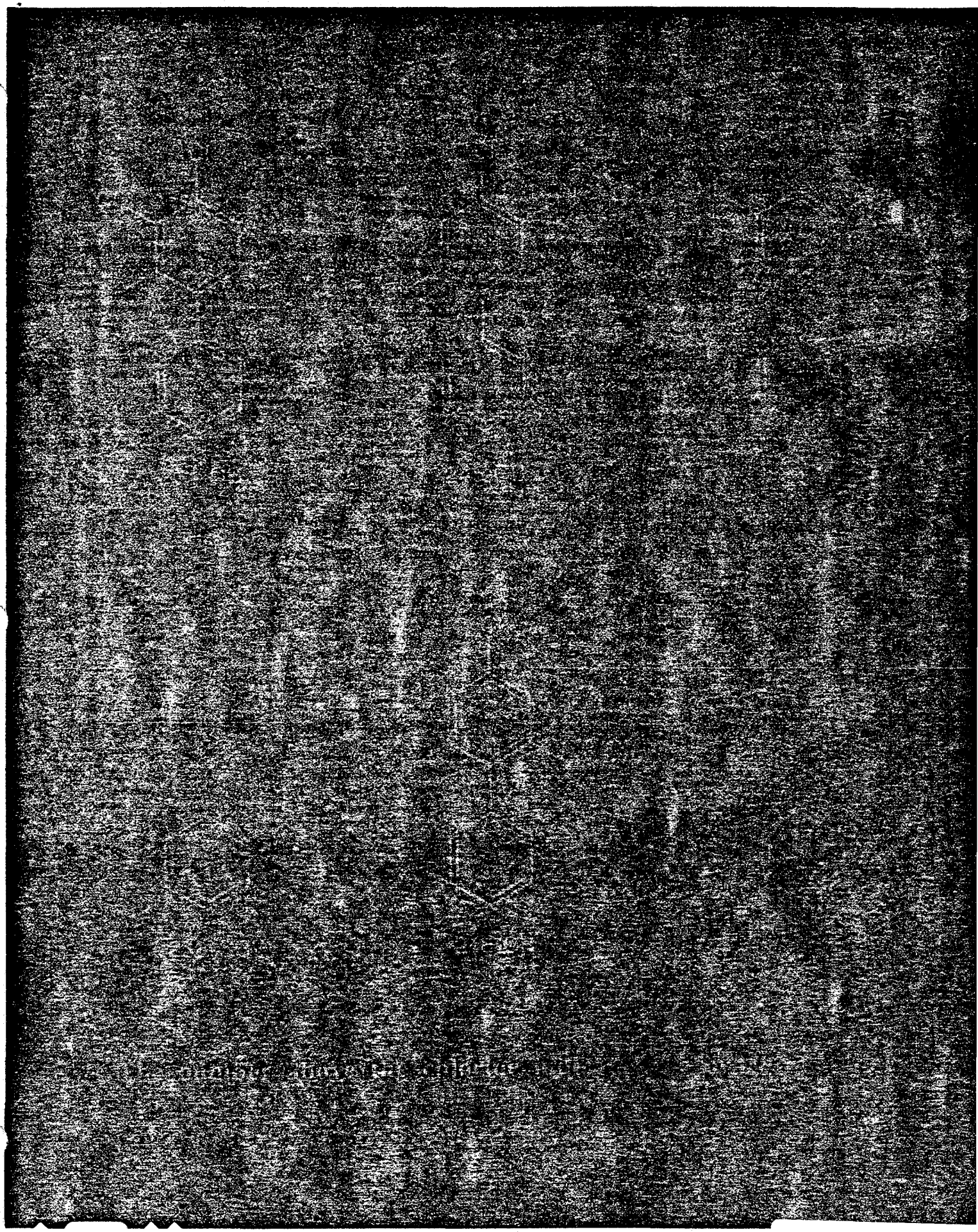
- Lab Results

Stepwise Dechlorination of PCBs with Hudson River Sediments

Distribution of tri-CB Dechlorination Products Different Sediments



Woods Pond	96%	3%	1%
Silver Lake	14%	79%	7%
Hudson River	63%	34%	3%



Concentrations of Planar PCB Congeners (Dioxin-like toxicity)

Congener* ↑ outer Cl. / inner Cl.	Conc. of Congener in Aroclor 1242 (%)	Conc. of Congener in Dechlorinated** Aroclor 1242 (%)	% Reduction
34-34	0.14	<0.005	>96.5
345-34	0.003	0.0005	83
345-345	0.0015	<0.00015	>90
245-34	0.33	0.068	80
234-34	0.43	0.061	86
2345-34	0.008	0.0043	46

avg. ≈ 80%

* Most active congeners in AHH/EROD enzyme indication assays

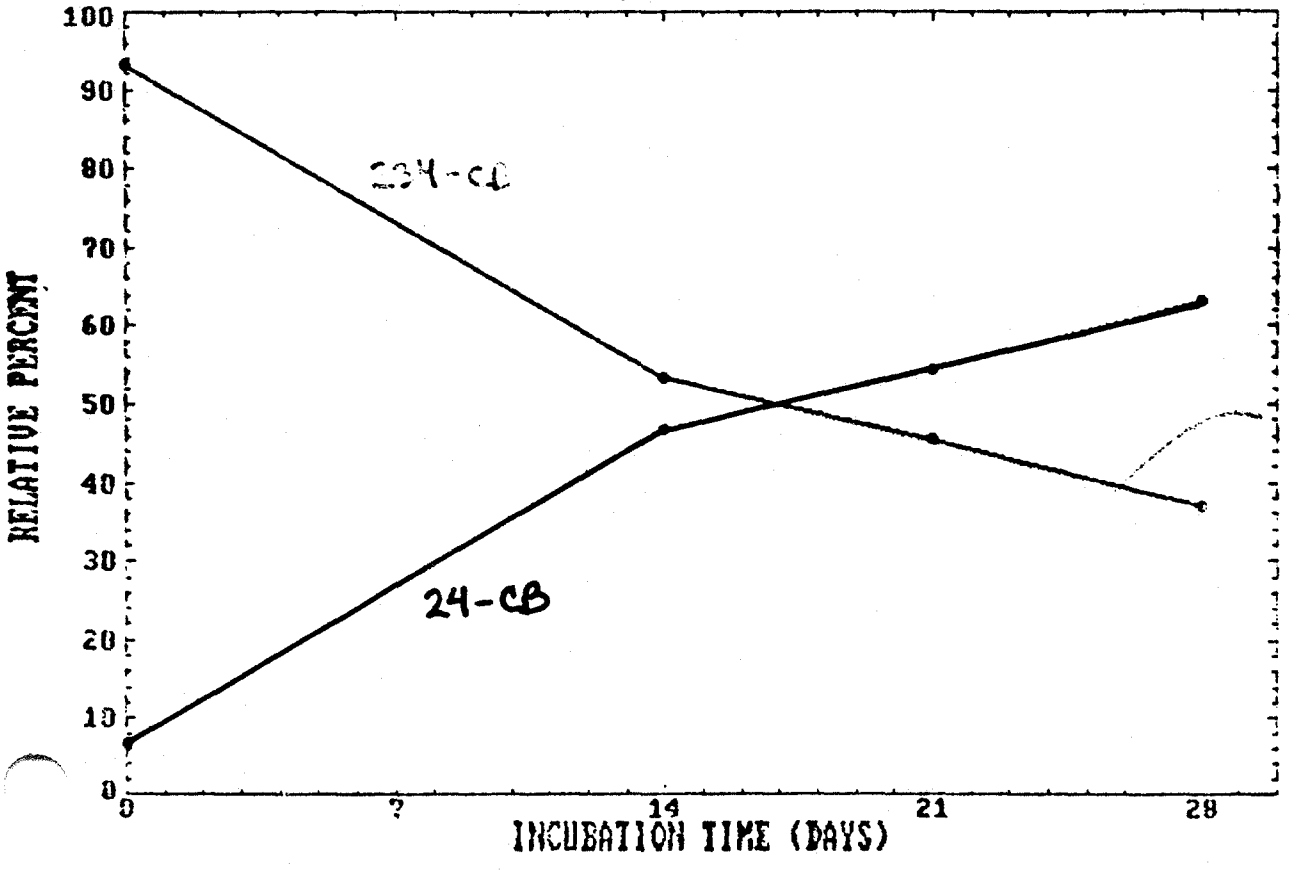
** Microbial dechlorination pattern C with Hudson River sediments

EROD assay demonstrated 75% reduction in enzyme induction with dechlorinated Aroclor 1242

Transfer Experiments onto Different Supports

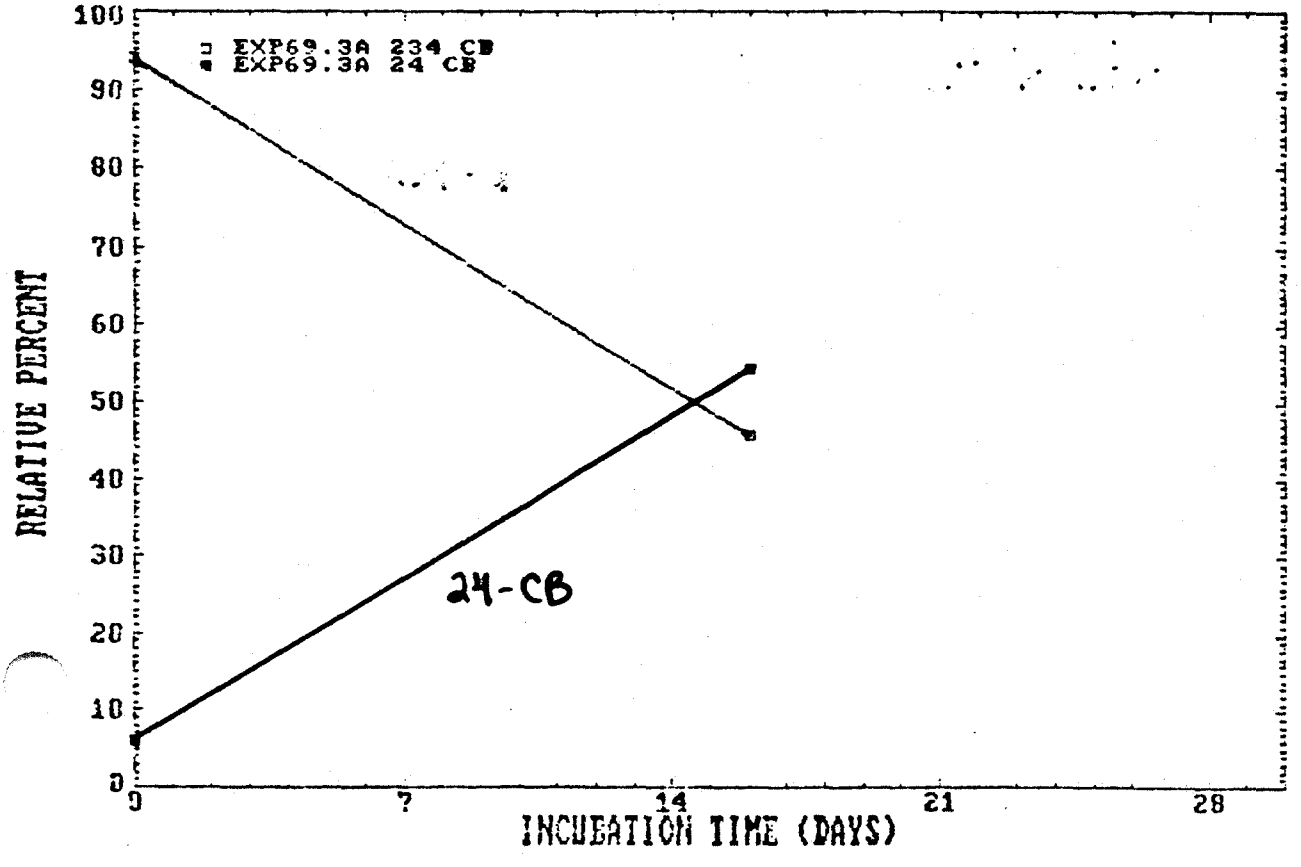
	#Cl removed/25 wks
No support	0.0
Sand	0.0
Sawdust	0.0
Clay	0.0
Vermiculite	0.0
Whole sediment	1.3
Peat	0.6
Peat/Vermiculite	1.2
Peat/Clay	0.5

1st Transfer



1/5V active WP on 234-

Fresh RAMM WP Broth 234-CB on silic 350 µM



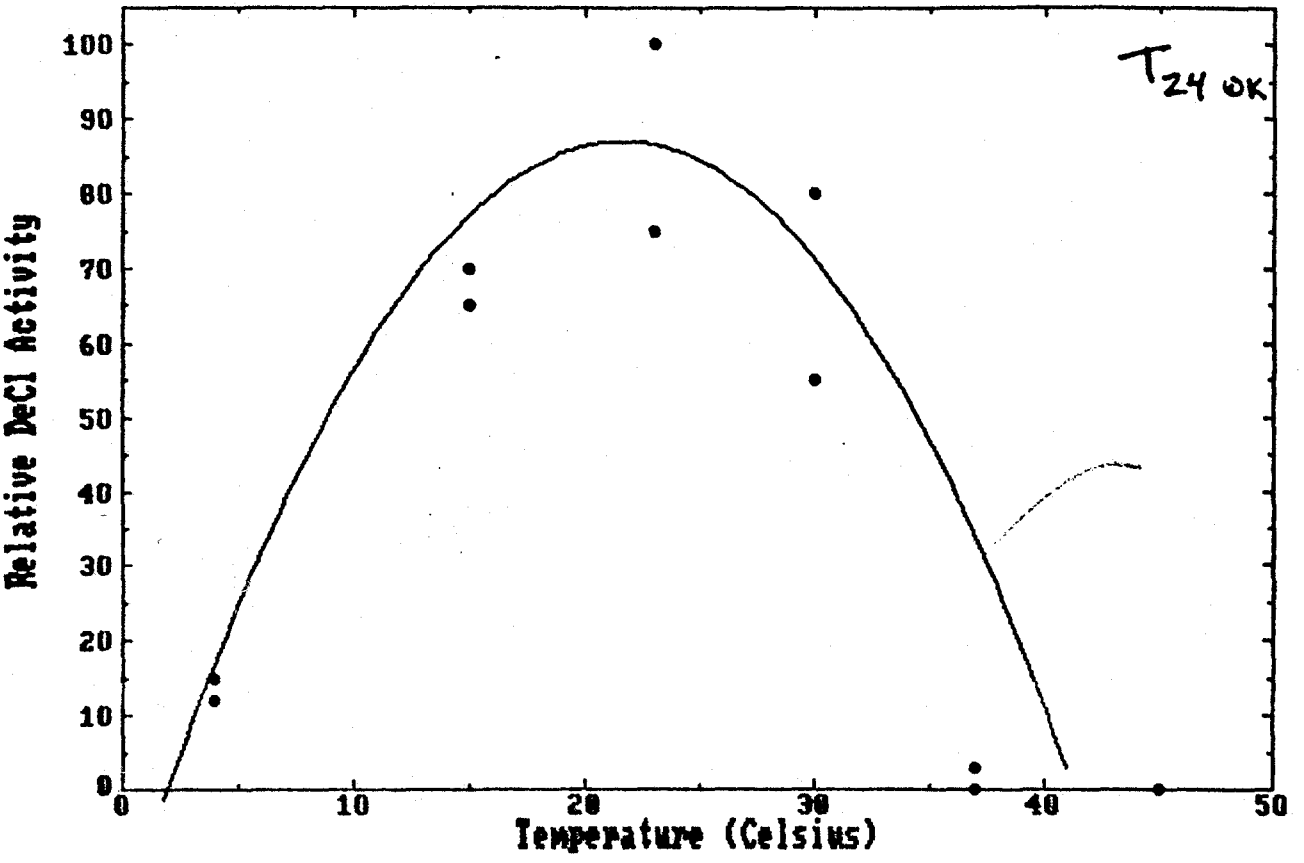
1/5V of above at 28 d

Fresh RA...
Autoclaved WP Sediment

234-CB on silic... 35 µM

402688

Early Temperature Effect Data



Temperature Profile:

Quensen & Tiedje:

- rate at 12°C ≈ ½ rate at 25°C
- Aroclor 1242

Temperature Effects

- Hudson River Sediments
- PCB = Aroclor 1242

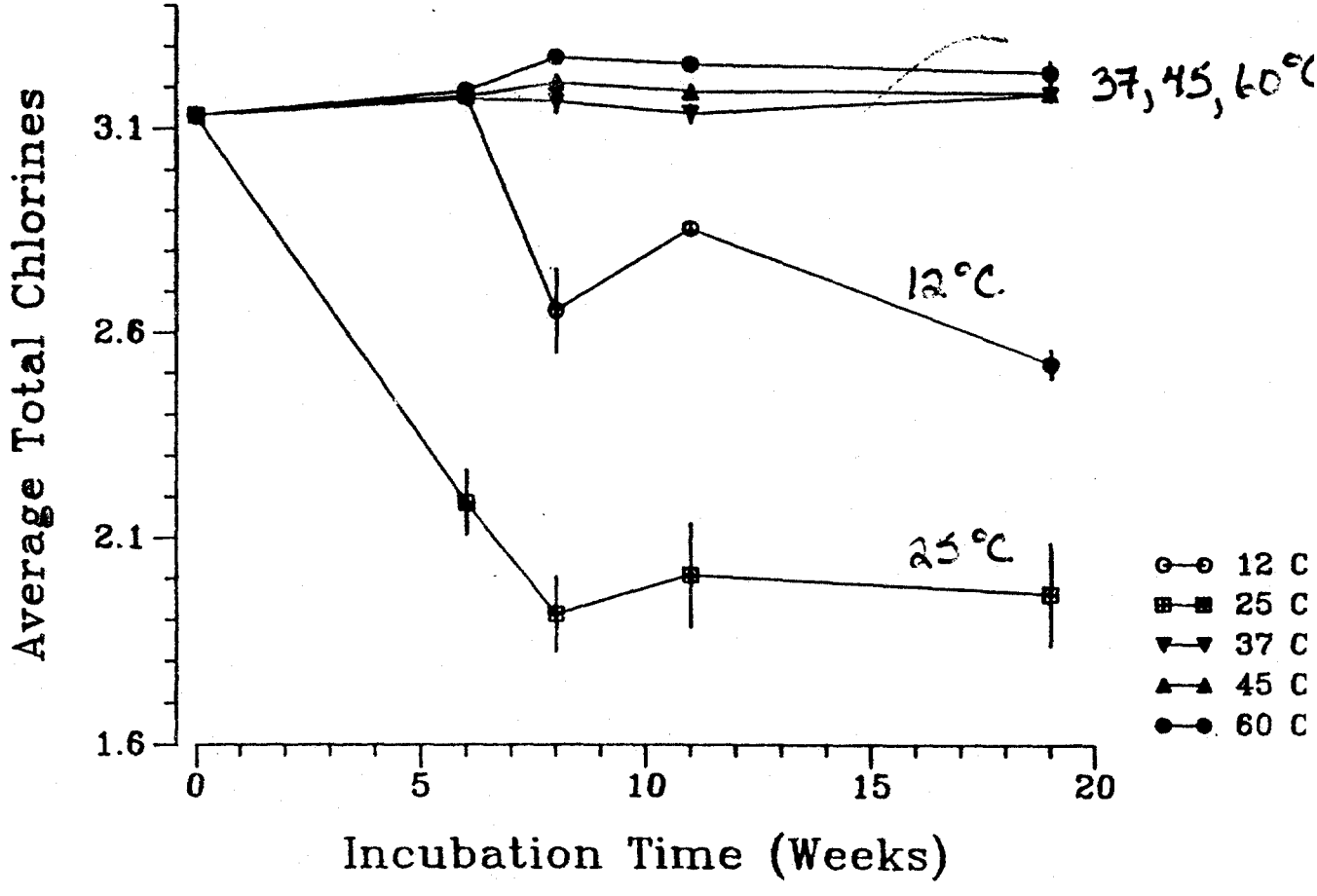
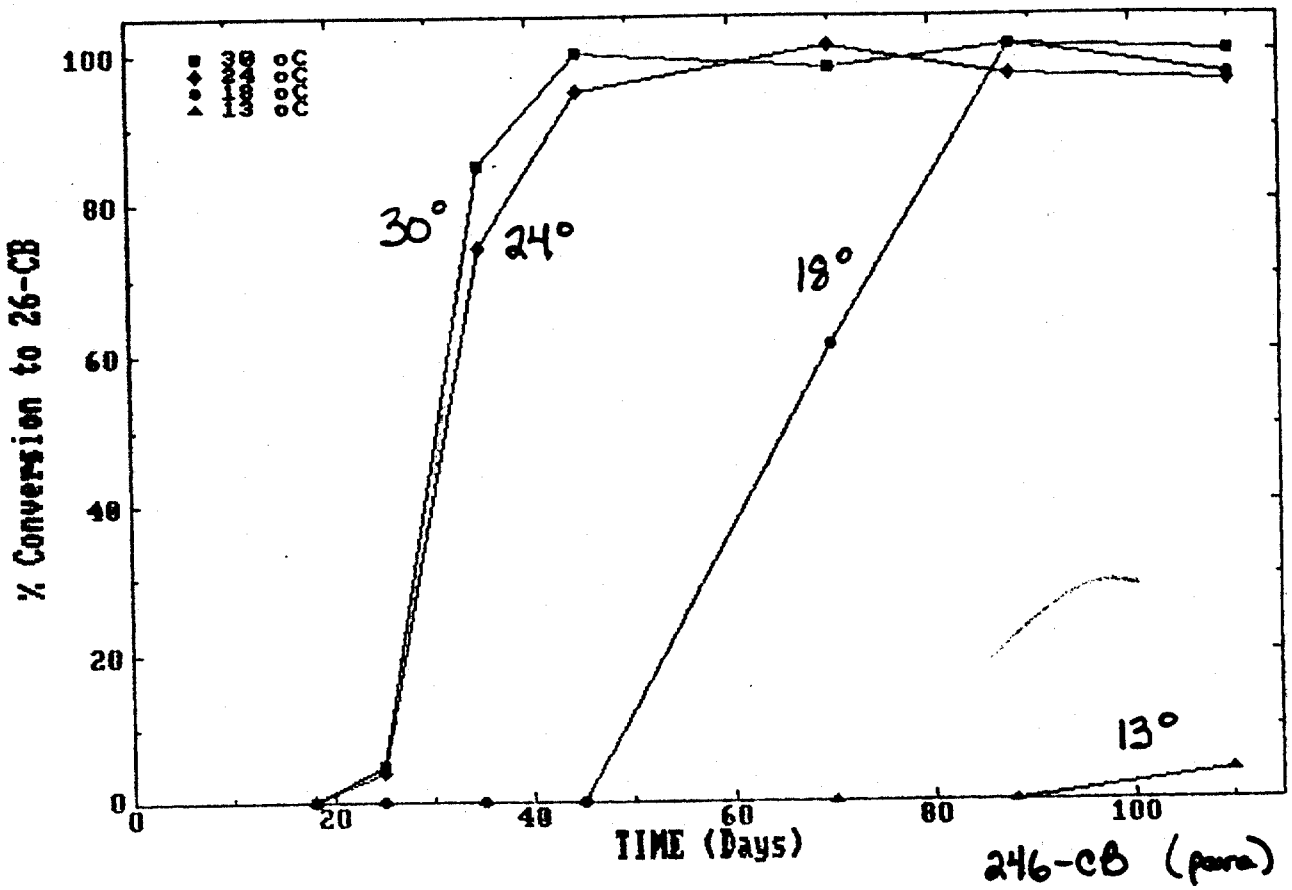
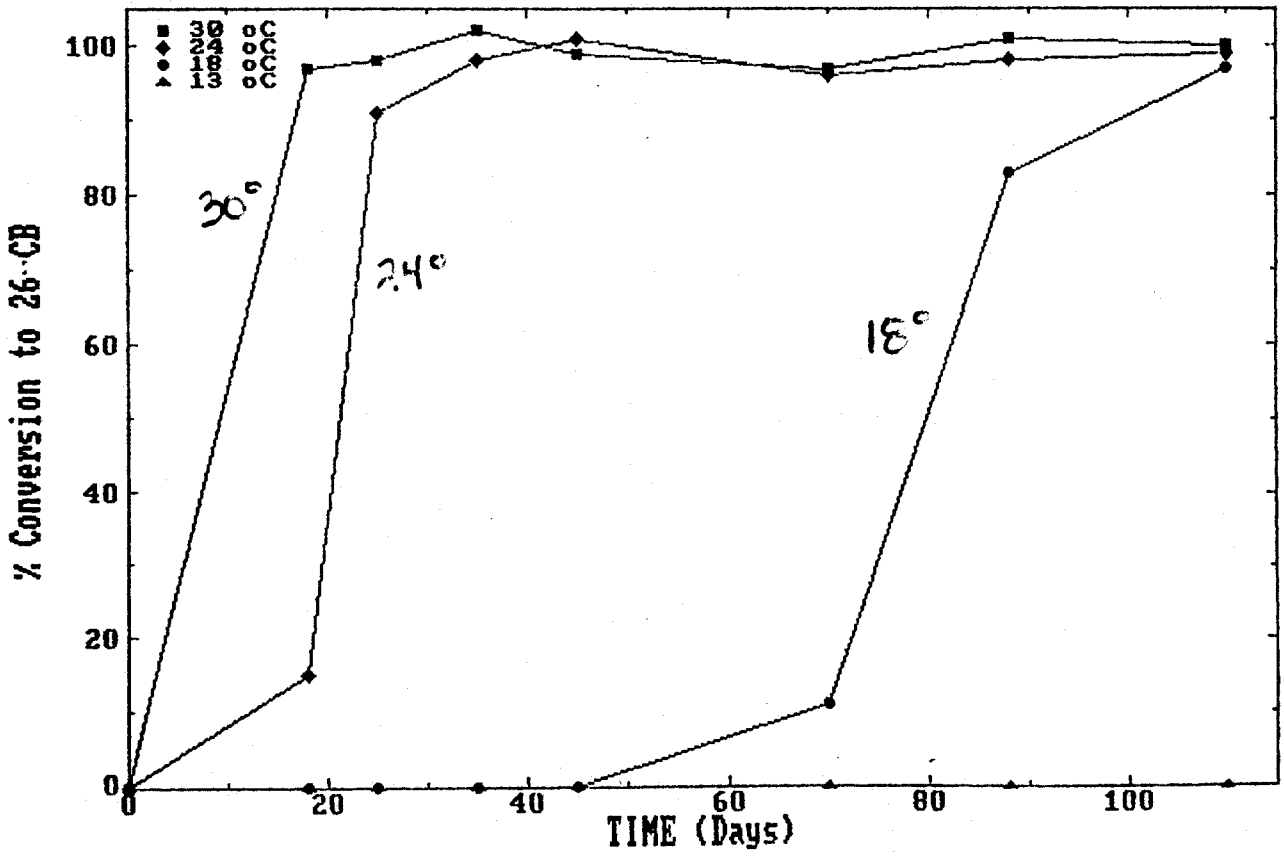


Figure 5-3. Effect of incubation temperature on the dechlorination of Aroclor 1242.

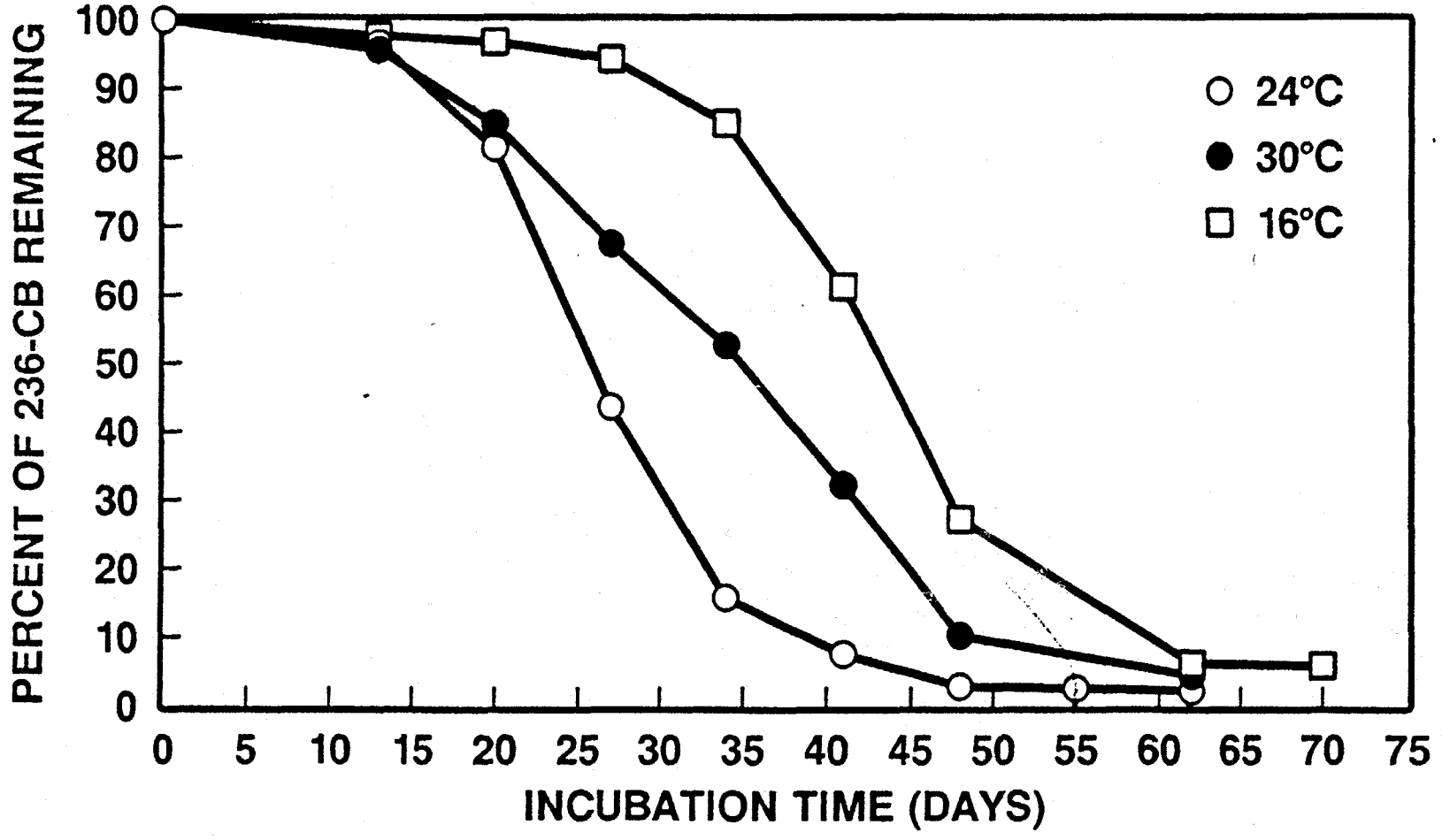


246-CB (para)

236-CB (meta)



EFFECT OF INCUBATION TEMPERATURE ON DECHLORINATION OF 236-CB IN CULTURES OF WOODS POND SEDIMENT



402693

Aerobic Degradation

- Introduction
- rDNA Efforts
- Dragstrip

Anaerobic Dechlorination

- Introduction
- Aroclors
- Single Congener

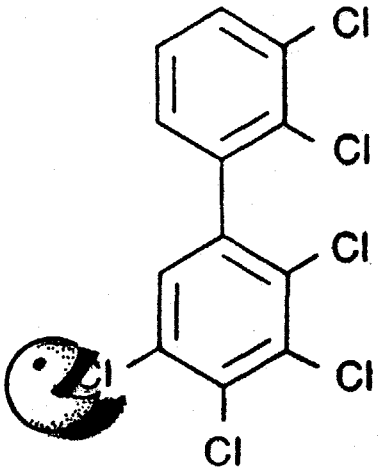
Anaerobic/Aerobic

- Lab Results

BACTERIAL TRANSFORMATION OF PCBs

	AEROBIC BACTERIA	ANAEROBIC BACTERIA
Organisms	*Pseudomonas, Alcaligenes, Corynebacterium, ...*	Unknown
Location	Water Column, Surface Sediments	Sub-sediments
Requirement for Activity	Oxygen	Lack of Oxygen
Type of Transformation	Oxidative Ring Cleavage	Reductive Dechlorination
Best Stubstrates	Mono- to Penta-CB	Tri- to Octa-CB

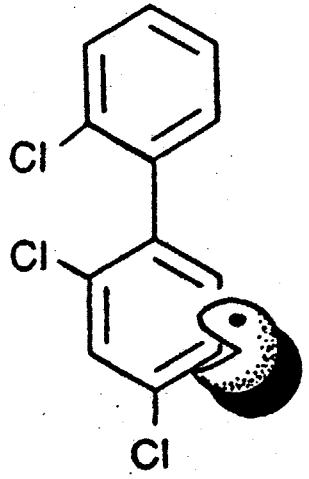
STEP #1



Anaerobic Dechlorination

Highly Cl

STEP #2

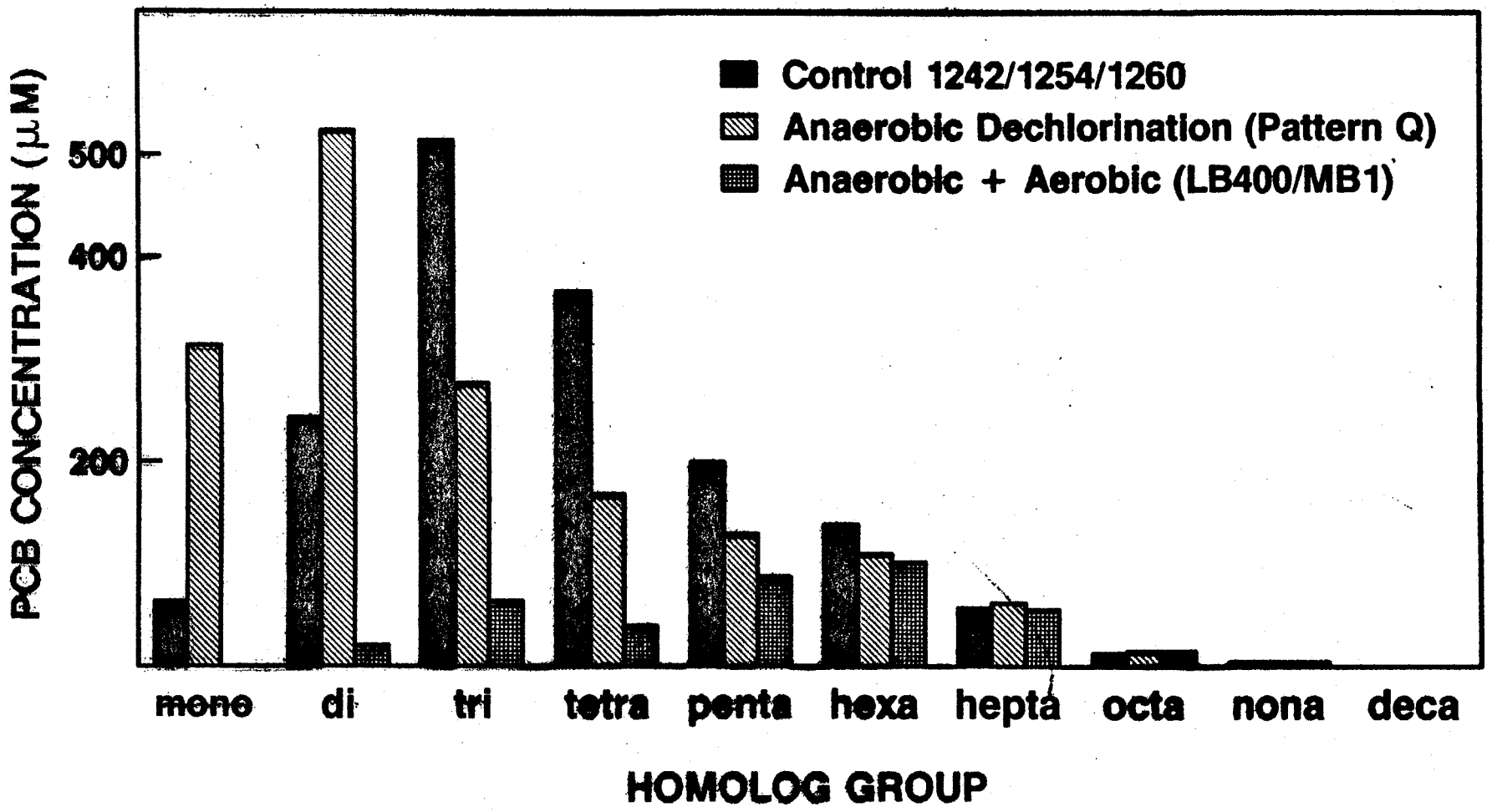


Aerobic Destruction

Lightly Cl

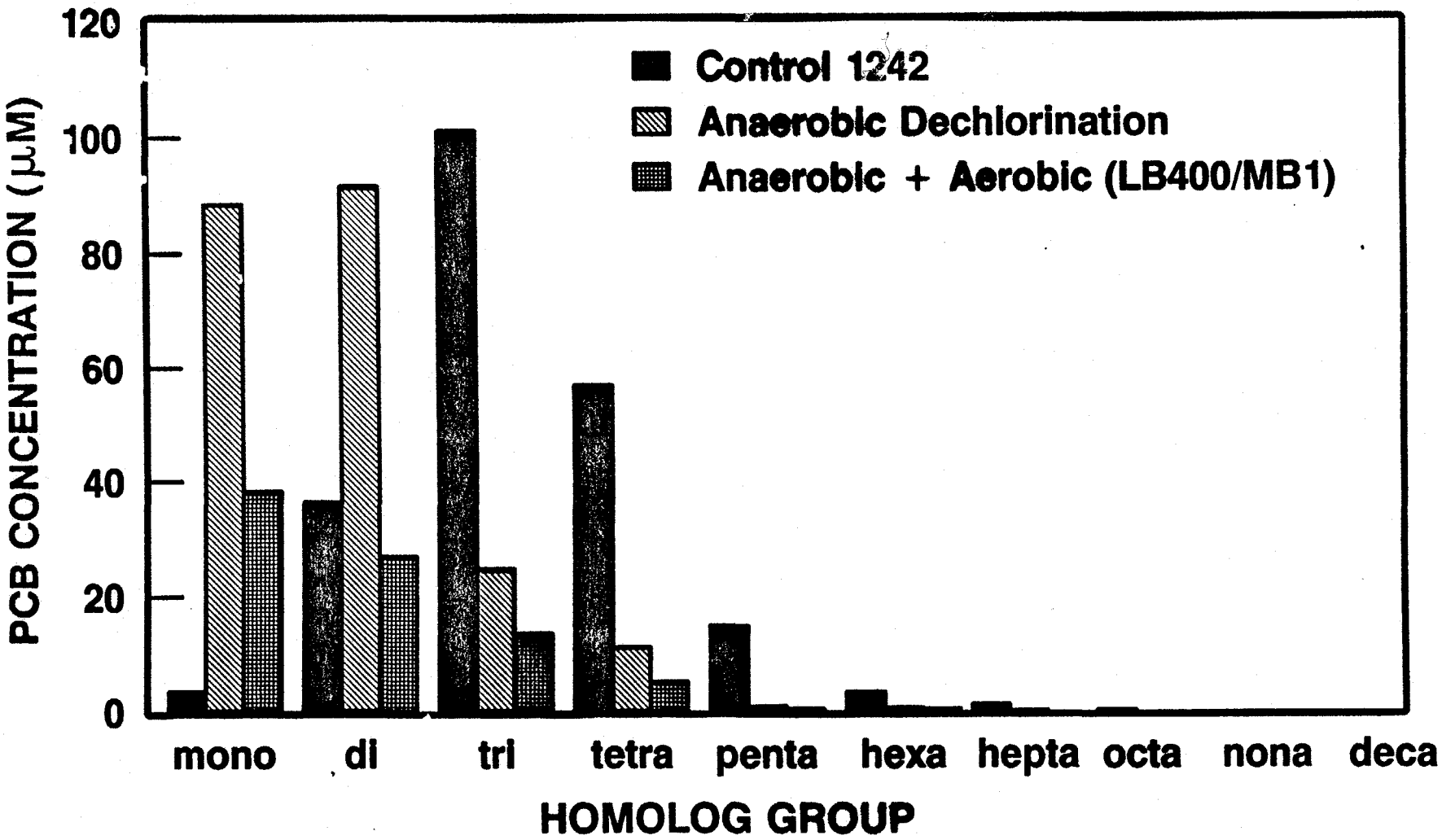
Sequential Anaerobic / Aerobic Treatment

- HR sediment spiked with Aroclor 1242/1254/1260
- [PCB] ↓ 85%; *m/p ce ↓



Sequential Anaerobic / Aerobic Treatment

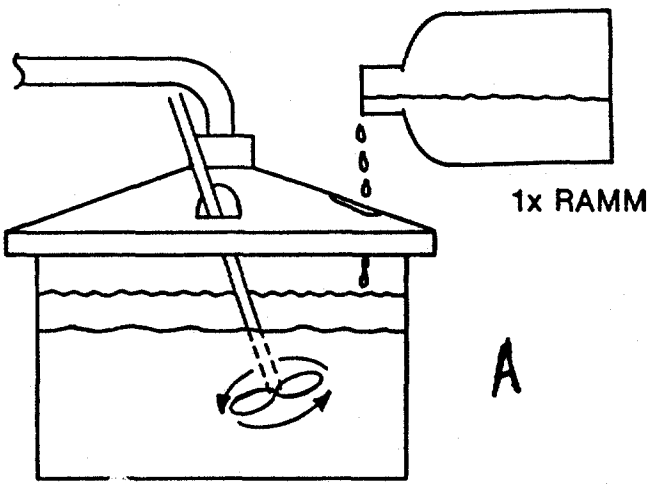
- Endogenous HR PCBs
- Anaerobic step done in environment

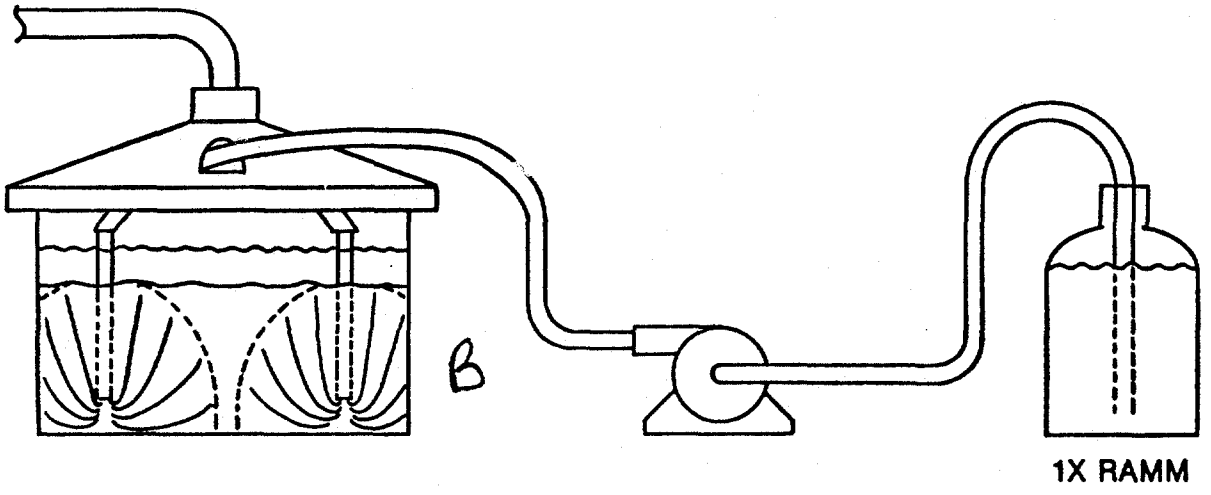


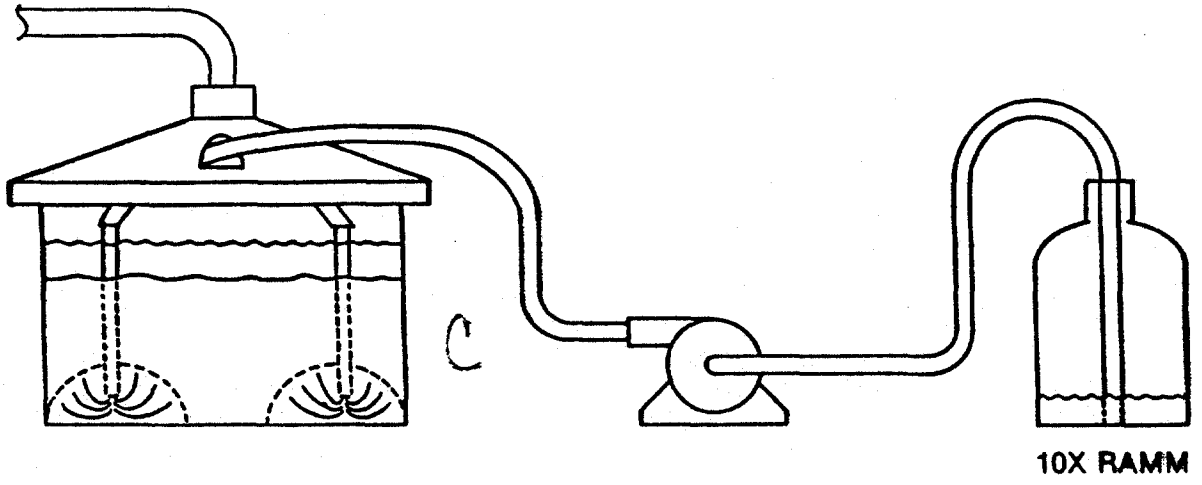
402699

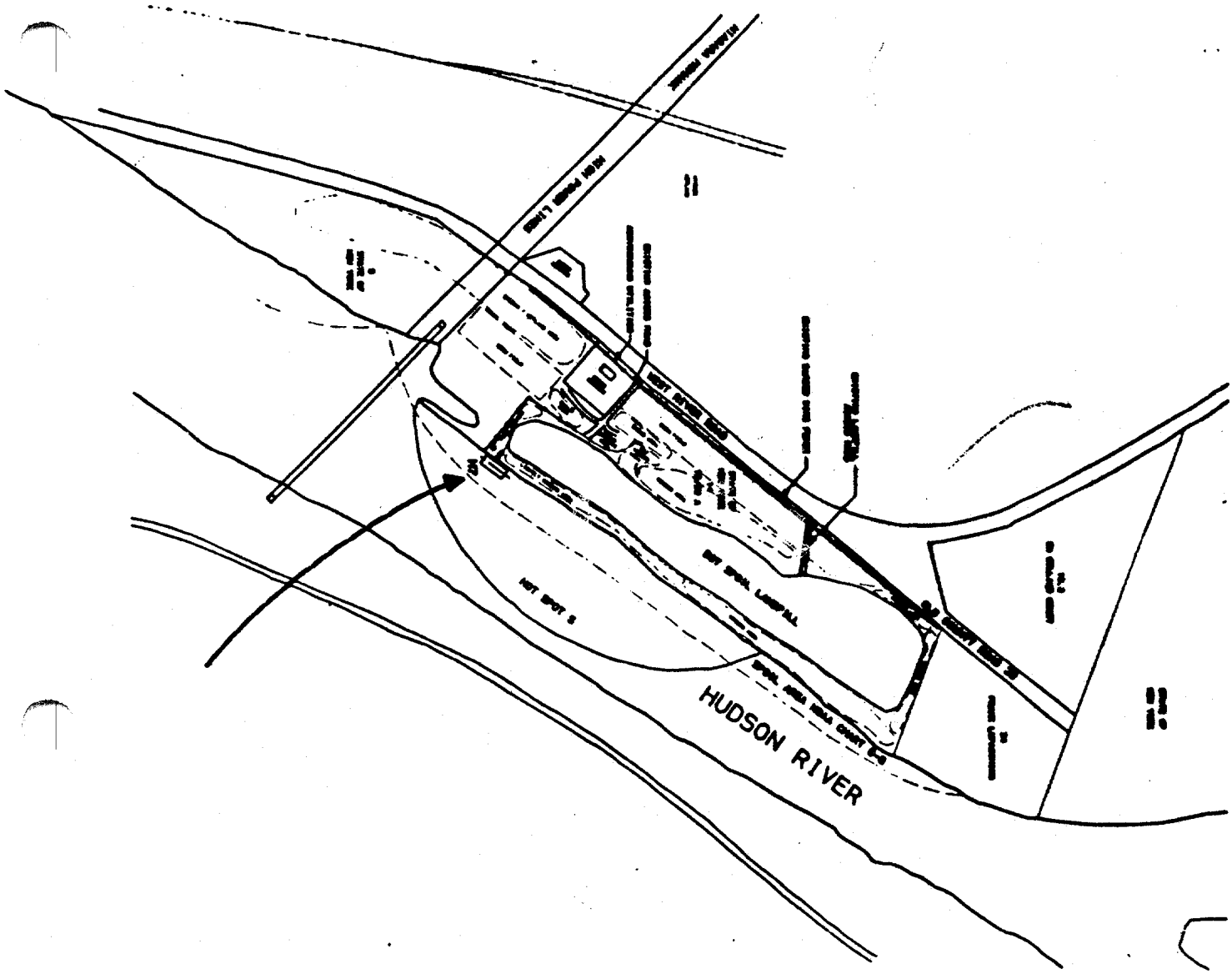
River Models (CRD)

T_8 A,C
uniform deCl



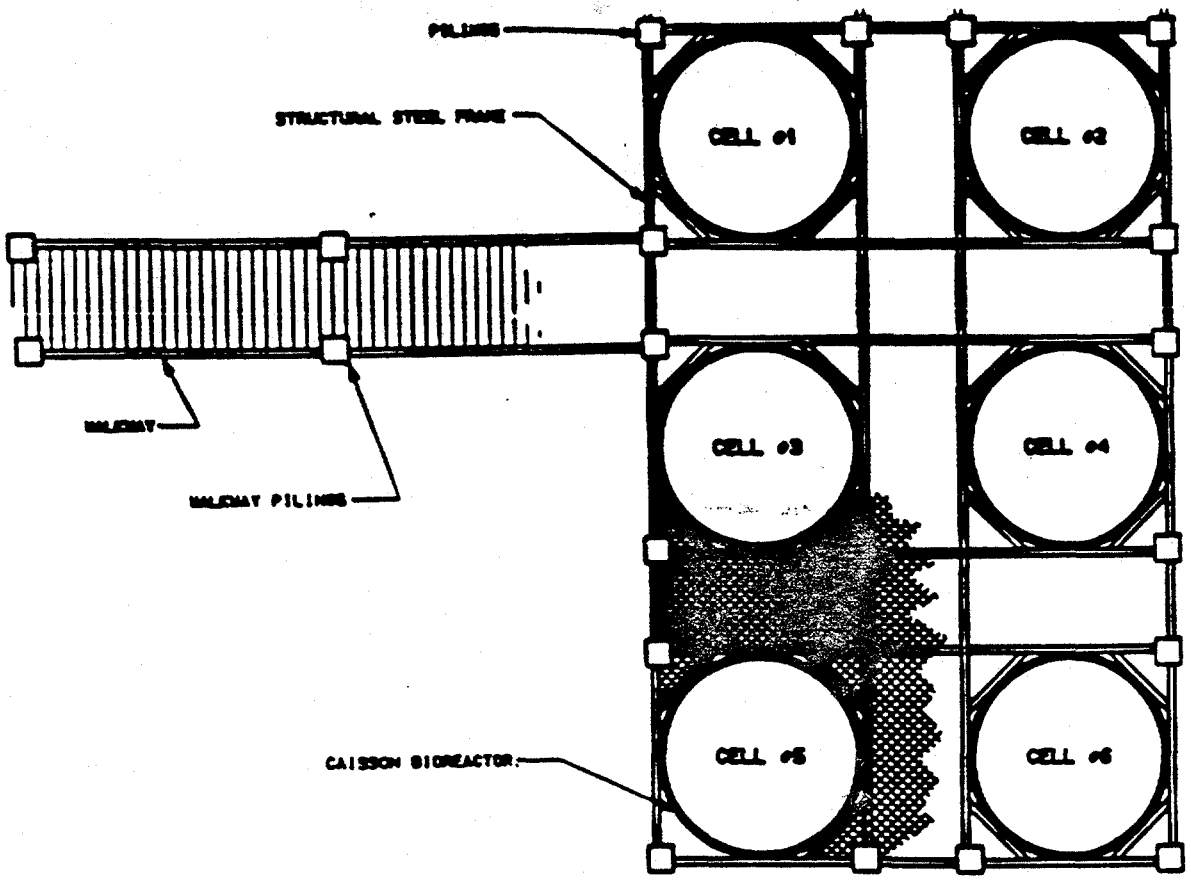






H7 Site

402701


PILINGS
STRUCTURAL STEEL FRAME
CELL #1
CELL #2
CELL #3
CELL #4
CELL #5
CELL #6
WALKWAY
WALKWAY PILINGS
CAISSON BIOREACTOR

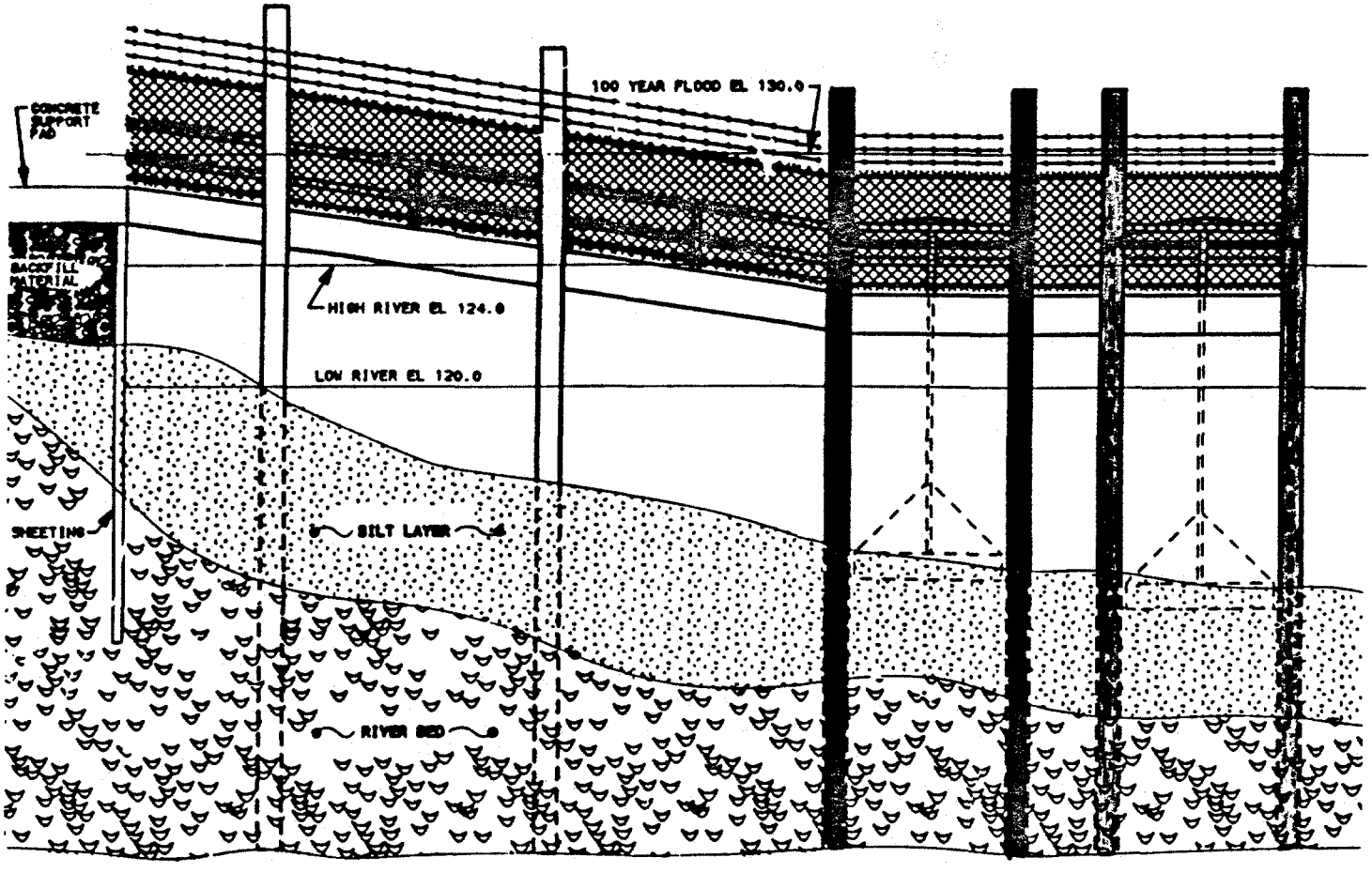

100 YEAR FLOOD EL 130.0
CONCRETE SUPPORT PAD
BACKFILL MATERIAL
HIGH RIVER EL 124.0
LOW RIVER EL 120.0
SHEETING
SILT LAYER
RIVER BED

FIGURE 1

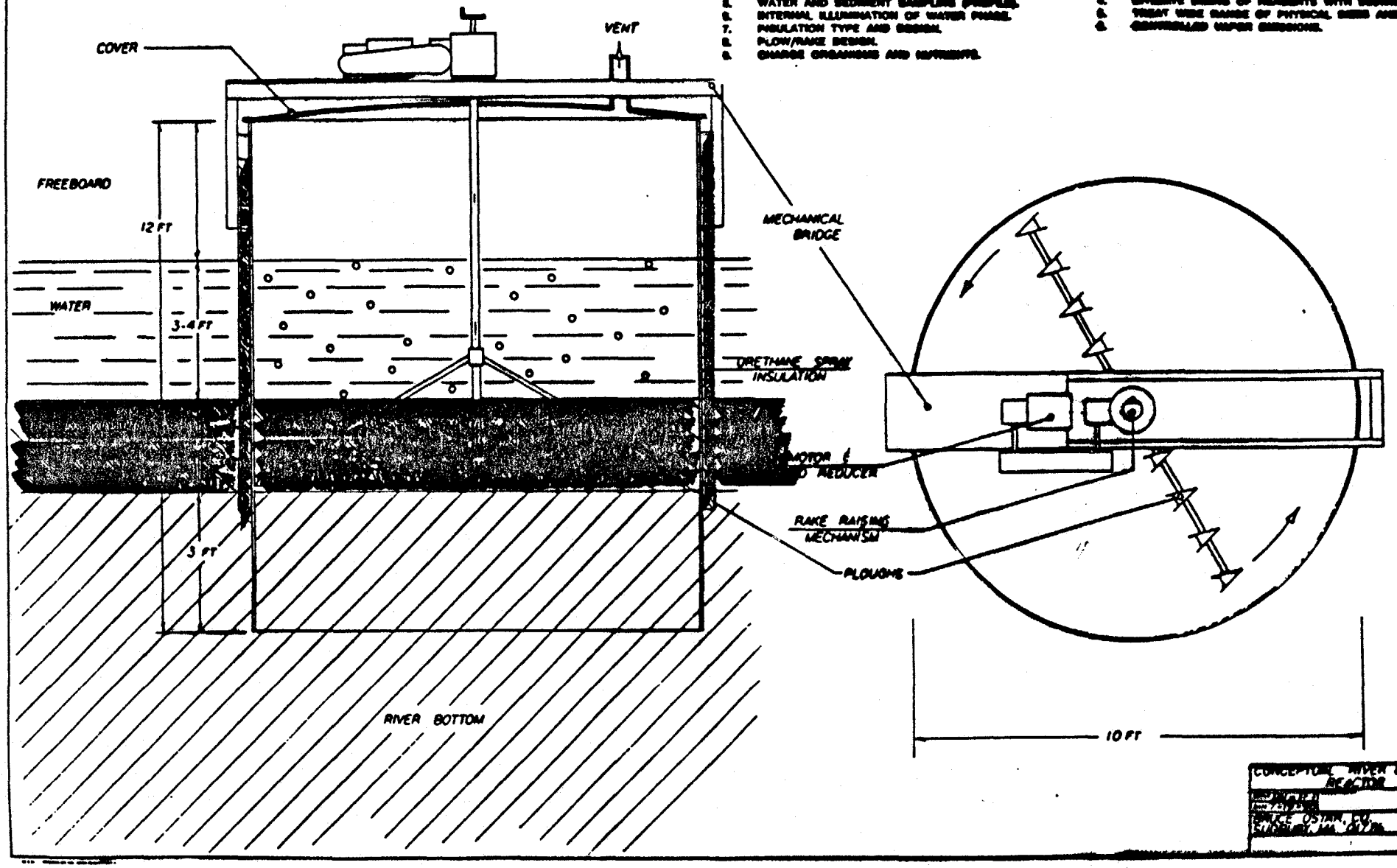
Current Design

DESIGN ISSUES

1. TEMPERATURE MEASUREMENT AND CONTROL.
2. HEATING SYSTEMS DESIGN.
3. AIR SUPPLY AND DISPERSION.
4. TREATMENT OFF-GASES.
5. WATER AND SEDIMENT SAMPLING (PROFILE).
6. INTERNAL ILLUMINATION OF WATER PHASE.
7. INSULATION TYPE AND DESIGN.
8. PLOW/RAKE DESIGN.
9. CHARGE ORGANICS AND NUTRIENTS.

DESIGN BASES

1. 50 CU. YDS. CAPACITY OF CONTAMINATED SEDIMENT, ONE TO TWO FEET DEEP.
2. CONTROLLED OPERATING TEMPERATURE (25 TO 35°C).
3. 24 HOUR OPERATION UNATTENDED.
4. EFFICIENT MIXING OF REAGENTS WITH SEDIMENTS.
5. TREAT WIDE RANGE OF PHYSICAL SIZES AND TYPES.
6. CONTROLLED VAPOR EMISSIONS.



CONCEPTUAL RIVER DIVERSION REACTOR

Estimated Time Schedule

March 1, 1991	Final hardware design and experimental plan Hardware assembly begins at fabricator shop
April 1, 1991	Site preparation begins
May 1, 1991	Site preparation completed Hardware assembly completed Construction of facility begins
June 15, 1991	On-site construction completed *In situ* test begins
September 15, 1991	*In situ* testing completed Site closure begins
October 15, 1991	Site closure completed

Estimated Protocol

Cell 1: Isolation only (control).

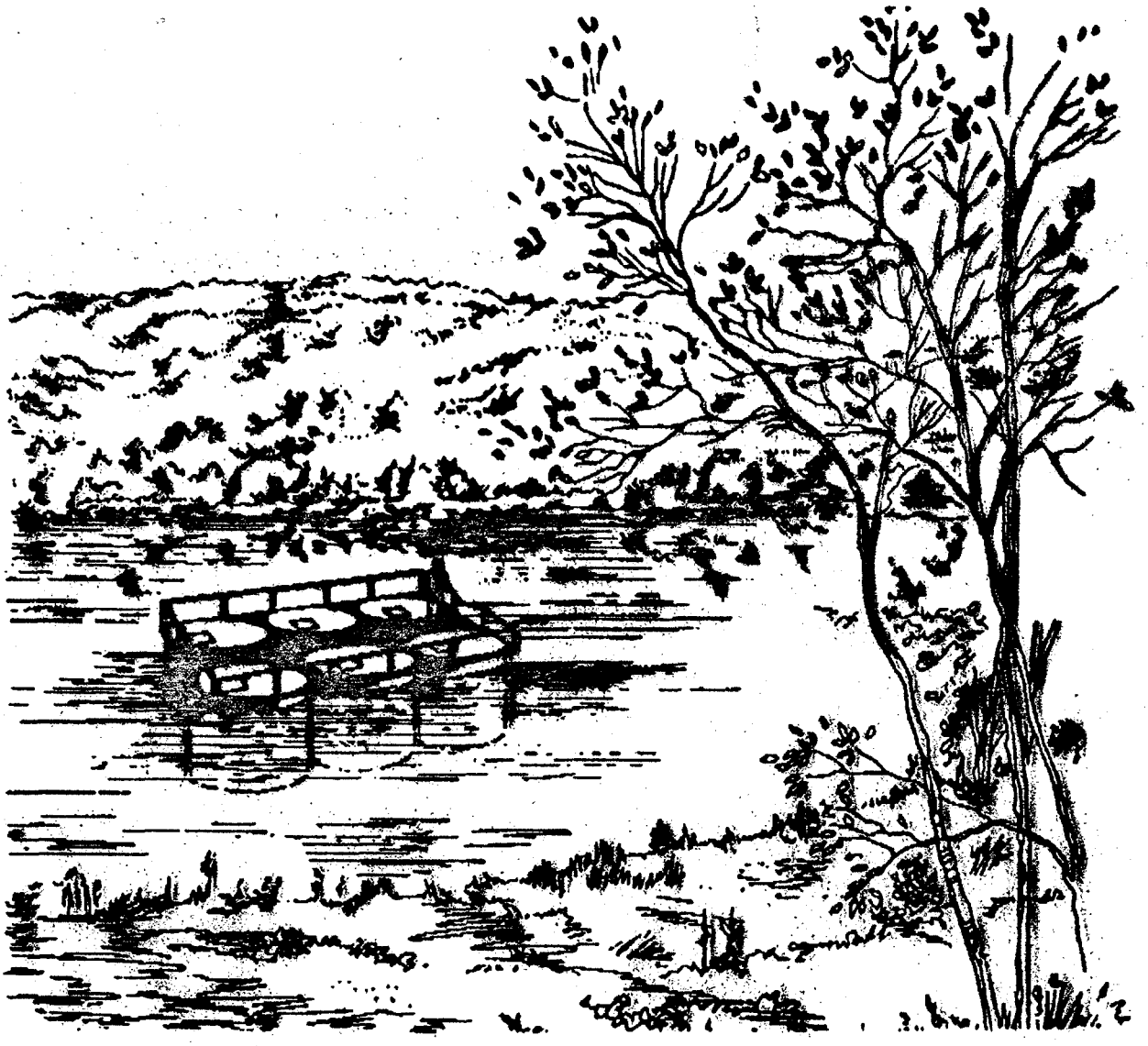
Cell 2: Nitrogen sparging and rake agitation only (control). No nutrient or microbial additions.

Cell 3: Full process control with added natural microorganisms cultured in the laboratory (e.g., pH, temperature, oxygen level, nutrient composition, agitation, and cell density).

Cell 4: Full process control with added indigenous microorganisms from the site cultured in the laboratory (see Cell 3).

Cell 5: Partial process control with added natural microorganisms (e.g., ambient temperature and pH, limited nutrients, agitation, aeration, and cell density).

Cell 6: Partial process control with indigenous microorganisms (see Cell 5).



Drawing shows proposed 10-by-25-foot experimental station to be placed in Woods Pond in Lenox. Pond is actually 230 acres, and the final project may include a 30-foot ramp from shore.

The Berkshire Eagle
April 4, 1990

Important New Findings:

- **Rates at lower temperatures ~room temperature rates**
- **Transfer/enrich organisms**
- **Organisms are common**
  - **Contaminated sediments**
  - **Uncontaminated sediments**
- **Potential for complete anaerobic degradation**
  - **Hudson River organisms can remove <u>all</u> outer chlorines**
  - **Housatonic River organisms can remove inner chlorines**

SUMMARY

- PCBs do biodegrade
  - widespread activity in sediments
  - aerobic
  - anaerobic
  - [PCB] in sediment/bass
- Attractive alternative
  - in-place
  - natural process
  - less invasive
- Moving forward
  - accelerate this natural process
  - lab scale
  - river model
  - site test in the river (1991)

PCB PROGRAM PERSONNEL -- HISTORICAL LIST

AEROBIC DEGRADATION

Organism Isolation:
Donna L. Bedard
Lawrence H. Bopp
Michael J. Brennan, Jr.
Carl Johnson
John H. Lobos
Ronald Unterman

Biochemistry/Pathways:
Donna L. Bedard
Lawrence H. Bopp
Michael J. Brennan, Jr.
John F. Brown, Jr.
Marie L. Haberl
Ralph J. May
Ronald Unterman
Robert E. Wagner
(University of Iowa)
(University of Kentucky)

Application/Scale-up:
Angelo A. Bracco
Ronald E. Brooks
Kenneth M. Carroll
David K. Dietrich
Mark R. Harkness
John B. McDermott
David P. Mobley
Charles Schwartz
Gregory L. Warner

Molecular Genetics:
Bruce D. Erickson
Frank J. Mondello
James R. Yates

Metabolism:
John A. Bergeron
Bruce D. Erickson
Kenneth M. Fish
David W. Krueger
David T. Lin

ANAEROBIC DECHLORINATION

Hudson River:
Daniel A. Abramowicz
Michael J. Brennan, Jr.
Edie L. Gallagher
Chitra Stokes
Heidi M. Van Dort
William A. Williams
(Michigan State University)
(New York Univ. Medical Center)
(Oregon State University)
(Stanford University)
(State Univ. of New York, Syracuse)
(University of Georgia)

Woods Pond:
Donna L. Bedard
Stephen C. Bunnell
Heidi M. Van Dort

Models/Scale-up:
Mark L. Stephens
(Celgene Corporation)
(University of Michigan)

ENVIRONMENTAL ASSESSMENT

John F. Brown, Jr.
George M. Frame, II
Ralph J. May
(AccuStandard)