Title: Reductive Dechlorination of Polychlorinated Biphenyls by Anaerobic Microorganisms from Sediments

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ABSTRACT

Microorganisms from Hudson River sediments reductively dechlorinated most polychlorinated biphenyls (PCBs) in Aroclor 1242 under anaerobic conditions. At 700 ppm Aroclor 53% of the total chlorine was removed in 16 weeks, and the proportion of mono- and dichlorobiphenyls increased from 16 to 88%. Dechlorination occurred primarily from the meta- and para- positions; ortho- only substituted congeners accumulated. These dechlorination products are both less toxic and more readily degraded by aerobic bacteria. These results indicate that reductive dechlorination may be an important environmental fate of PCBs, and suggest that a sequential anaerobic/aerobic biological treatment system for PCBs may be feasible.

Prior to the 1970's, polychlorinated biphenyls (PCBs) were widely used for a variety of industrial purposes including fluid filled capacitors and transformers, hydraulic fluids, heat transfer fluids, plasticizers, and carbonless copy paper. In general, PCBs are considered to be highly persistent in natural environments such as soils and sediments. Their biological degradation under aerobic conditions is generally limited to the lesser chlorinated congeners with at least two adjacent unsubstituted carbon atoms (*1*). Recently, however, the altered PCB congener distribution patterns found in anaerobic sediment samples from the upper Hudson River have been interpreted to be the result of biologically mediated reductive dechlorination (*2,3*).

While the main PCB input to the upper Hudson River is reported to have been Aroclor 1242 between 1951 and 1973, subsurface sediment samples now show depletion of the tri- and higher chlorinated congeners present in Aroclor 1242 and a corresponding increase in the proportion of ortho- only substituted mono- and dichlorobiphenyls (*2,3*). Similar but generally less pronounced differences between known PCB inputs and analyzed sediment samples taken several years later have also been observed for Waukegan Harbor (Illinois) (*4*), and Silver Lake (*3*) and the Acushnet River (both in Massachusetts) (*5*). In all of these cases a biological process was presumed to be responsible for the difference in congener distribution pattern because congener selectivity was observed and a strictly abiologic reduction of PCBs is not possible in these anaerobic sediments (*6*). Attempts to chemically dechlorinate aromatic compounds under similar conditions using reduced iron-porphyrins have not

succeeded (7). However, others have suggested that the observed enrichment of mono- and dichlorobiphenyls in the Hudson River sediments is the result of selective partitioning (8). Thus laboratory experiments to directly demonstrate biological dechlorination of PCBs are important to clarify the mechanism. We here report the first successful demonstration of biologically mediated reductive dechlorination of an Aroclor mixture.

We assessed the ability of microorganisms from PCB contaminated Hudson River sediments (60 to 562 ppm PCBs) (9) to dechlorinate Aroclor 1242 under anaerobic conditions by eluting microorganisms from the contaminated sediments and transferring them to a slurry of reduced anaerobic mineral medium (10) and noncontaminated sediments (11) in tightly stoppered serum bottles (12). This reduced the PCB background enabling us to quantify the dechlorination of freshly added Aroclor 1242. Three concentrations of Aroclor were used corresponding to 14, 140, and 700 ppm on a sediment dry weight basis.

Dechlorination of the Aroclor by the eluted organisms was evident from a simple visual inspection of the chromatograms of PCBs extracted after 16 weeks of incubation (Figure 1). Early eluting peaks, corresponding to the lesser chlorinated congeners, increased with time in the live samples but not in the autoclaved controls. There was a corresponding decrease in the later-eluting, more highly chlorinated congeners. Most notable was the accumulation of chlorobiphenyls substituted only at the ortho positions. 2-Chlorobiphenyl (2-CB) increased from 0 to 63% of the total PCBs in the live treatments receiving 700 ppm Aroclor, and 2,2'-CB and/or 2,6-CB (coeluting isomers) increased from 1 to 14%. 2,2',6-CB also increased from 0.4 to 2%.

The progressive nature of the dechlorination process was evident from changes in the relative proportions of mono-, di-, tri-, tetra-, penta-, and

hexa-CBs at each sampling time (Table 1). Aroclor 1242 contains predominantly tri- and tetra-CBs; these were progressively dechlorinated to di- and mono-CBs in the live treatments receiving 140 and 700 ppm Aroclor while there was no appreciable change over time in the autoclaved controls.

PCB dechlorination in the live treatments occurred primarily from the meta and para positions with no significant loss of chlorines in the autoclaved controls (Figure 2). Dechlorination was most extensive at the highest PCB concentration. In the 700 ppm treatment the meta plus para chlorines per biphenyl decreased from an average of 1.98 to 0.31 after 16 weeks, but decreased to only 1.19 in the 140 ppm treatment (*13*). At 14 ppm there was no observable difference between the live and autoclaved controls after 16 weeks. The dependence of dechlorination on PCB concentration may be related to PCB bioavailability. Higher concentrations in the sediment would result in higher solution concentrations, and it is probably only the PCBs in solution that are available for uptake by the dechlorinating microorganisms (*14*). Meta and para chlorines were estimated together because it was not always possible to distinguish the two classes when coeluting isomers were involved. It is evident that dechlorination of both positions occurred because meta and para chlorines occur in nearly equal proportions (5:4 ratio) in Aroclor 1242. The observed decrease in meta plus para chlorines from 1.98 to 0.31 cannot be accounted for unless dechlorination occurred at both positions.

The experiment described above has also been performed using microorganisms from a non-contaminated site in the Hudson River (*11*). No dechlorination of 700 ppm Aroclor 1242 was observed in 16 weeks. Perhaps the long term exposure (>15 years) to high PCB concentrations at the contaminated

site selected for this dechlorinating activity. A deficiency of electron acceptors limits microbial growth in most anaerobic environments. Thus any microorganisms which could use PCBs as terminal electron acceptors would be advantaged in anaerobic sediments. It is also conceivable that they may obtain energy from the dechlorination step itself. By analogy, the 3-chlorobenzoate dechlorinating strain DCB-1 may derive energy from the exergonic aryl dechlorination ($\Delta G' = -112$ KJ/mol) as the growth yield for the consortium is higher when grown on chlorobenzoate than on benzoate (*15*).

The contaminated Hudson River sediment used in this experiment was collected from site H7. The PCB congener profile observed in our laboratory experiment is very similar to the Pattern C profile previously described for environmental samples from this site (*2*). Thus it is probable that the indigenous sediment organisms that were responsible for the dechlorination products found in the laboratory also accounted for dechlorination of PCBs *in situ*.

The removal of meta and para chlorines, while not reducing the molar concentration of PCBs, can be expected to decrease the mammalian toxicity of the PCB residues and make them more readily degradable by aerobic bacteria. The PCBs with the greatest dioxin-like toxicity are those with at least one meta and a para chlorine on each ring and no more than one ortho chlorine (eg. 3,3',4,4'-CB, 2,3,3',4,4'-CB, 2,3',4,4',5-CB) (*16*). Removal of the meta and para chlorines from these congeners should eliminate their toxicity. Because of the potential of this process for detoxication, an assessment of PCB dechlorination should be made on a case by case basis before deciding on the appropriate remedial action for contaminated sediments.

The most commonly known initial step in the aerobic degradation of PCBs is catalyzed by a dioxygenase. This requires two adjacent unsubstituted carbons - at either the ortho and meta positions (2,3-dioxygenase) or meta and para positions (3,4-dioxygenase). Removal of the meta and para chlorines would therefore make any biphenyl ring subject to 3,4-dioxygenase attack, and any ring with no more than one ortho chlorine subject to 2,3-dioxygenase attack. The aerobic degradation of the PCB congeners accumulating in this experiment and in Hudson River sediments has been demonstrated by one or more strains of bacteria (*1*). Hence it is likely that all PCBs can be biodegraded by a suitable sequential anaerobic/aerobic process.

FOOTNOTES

1. D. L. Bedard, M. L. Haberl, R. J. May, and M. J. Brennan, *Appl. Environ. Microbiol.* 51, 761, (1986) and D. L. Bedard, R. E. Wagner, M. J. Brennan, M. L. Haberl, and J. F. Brown, Jr., *Appl. Environ. Microbiol.* 53, 1103 (1987); K. Furukawa, N. Tomizuka, and A. Kamibayashi, *Appl. Environ. Microbiol.* 38, 301 (1979).

2. J. F. Brown, Jr., *et al.*, *Northeast. Environ. Sci.* 3, 167 (1984).

3. J. F. Brown, Jr., *et al.*, *Environ. Toxicol. Chem.*, 6, 579 (1987) and *Science*, 236, 709 (1987).

4. D. L. Stalling, *Isomer Specific Composition of PCB Residues in Fish and Sediment from Waukegan Harbour and Other Great Lakes Fish* (Columbia National Fisheries Research Laboratory, Columbia, MO, 1982).

5. J. F. Brown, Jr., and R. E. Wagner, Abstract #136 of the Eighth Annual Meeting of The Society of Environmental Toxicology and Chemistry, Pensacola, Florida (1987).

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7. R. S. Wade, R. Havlin, and C. E. Castro, *J. Am. Chem. Soc.* 91, 7530 (1969), and G. M. Klecka and S. J. Gonsior, *Chemosphere* 13, 391 (1984).

8. B. Bush, L. A. Shane, M. Wahlen, and M. P. Brown, *Chemosphere* 16, 733 (1987).

9. Contaminated sediments were collected from near the west bank of the Hudson River at River Mile 193.3. This corresponds to site H7 in (*2*).

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11. noncontaminated sediments were collected upstream from the site in (*9*) at River Mile 215. Aroclor 1260-like residues were detected (1 to 2 ppm) but these did not interfere with the analysis of the Aroclor 1242 added or its dechlorination products.

12. Sieved air dried sediment from a noncontaminated Hudson River site (*11*) was added to each of 12 160 ml serum bottles. Reduced anaerobic mineral medium (RAMM) (*10*) and 50 ul of ethanol were added while flushing with filter sterilized O_2-free N_2/CO_2 (80:20, v:v), and the bottles sealed. The bottles were incubated until methane production was detected to indicate that anaerobic conditions were established. The microorganisms were eluted from contaminated Hudson River sediment (*9*) by shaking a slurry of equal volumes of sediment and RAMM and then allowing the slurry to settle for 15 min. Supernatant (50 ml) from this slurry was used to inoculate each serum bottle. Six of the 12 bottles, which served as controls, were autoclaved twice with a three day interval between. Aroclor 1242 in 100 ul of acetone was added in three different amounts (0.7, 7, and 35 mg per bottle) to give two live and two autoclaved bottles at each concentration. The bottles were sealed with Teflon lined stoppers after the Aroclor additions, and the bottles were shaken for 30 min after the PCB addition and for 10 min prior to each sampling event. Incubation was in the dark at 25C. Samples (approximately 2 ml of slurry) were removed with sterile pipets while flushing with filter sterilized O_2-free N_2/CO_2 (80:20, v:v), and bottles were resealed after sampling. The samples were frozen until extracted.

The samples were extracted once with 10 ml of acetone (to remove water) and twice with hexane:acetone (9:1, v:v) by shaking. The acetone was extracted with 2% NaCl in distilled water, and the hexane extract cleaned up using sulfuric acid, mercury, and Florisil following a clean up procedure slightly modified from EPA Method 608, *Federal Register*, 44, 69501 (1979). The samples were then analyzed on a capillary gas chromatograph equipped with a 25 m, 0.2mm i.d. HP-5 (Hewlett Packard) capillary column and electron capture detector.

The percentage of the total PCBs (on a molar basis) represented by each of 60 resolvable peaks was calculated, and data summarized by homolog class (mono-, di-, etc.), and the average number of ortho vs. meta plus para chlorines per biphenyl. Several simplifying assumptions were made in performing these calculations. For peaks with coeluting congeners with different numbers of chlorines, the proportion of each was estimated from the average molecular weight for that peak as determined by mass spectroscopy and provided by R. E. Wagner and J. F. Brown, Jr. For peaks with coeluting isomers it was assumed that they occurred in equal proportions. It was further assumed that all PCBs in the same peak increased or decreased to the same extent as a result of the dechlorination.

13. There is some variation in the estimate of the number of chlorines per biphenyl introduced by the extraction and clean up procedure. The number of meta plus para chlorines per biphenyl for Aroclor 1242 standards was 1.81 to 1.83, but varied between 1.73 and 1.93 for autoclaved controls at 700 ppm Aroclor 1242.

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18. We thank R. Unterman and J. F. Brown, Jr. for providing the Hudson River sediments and R. E. Wagner and J. F. Brown, Jr. for providing the congener specific analysis of Aroclor 1242 upon which our calibration method is based. This research was supported by grants from General Electric Company and the State of Michigan Research Excellence Fund. Published as Journal Article No. _____ of the Michigan Agricultural Experiment Station and of the Center for Environmental Toxicology.

Legend:

Table 1: Changes in PCB homolog distribution over time for the 700 ppm live treatment. Values are the means of two replicates ± the standard deviation of the mean.

Captions:

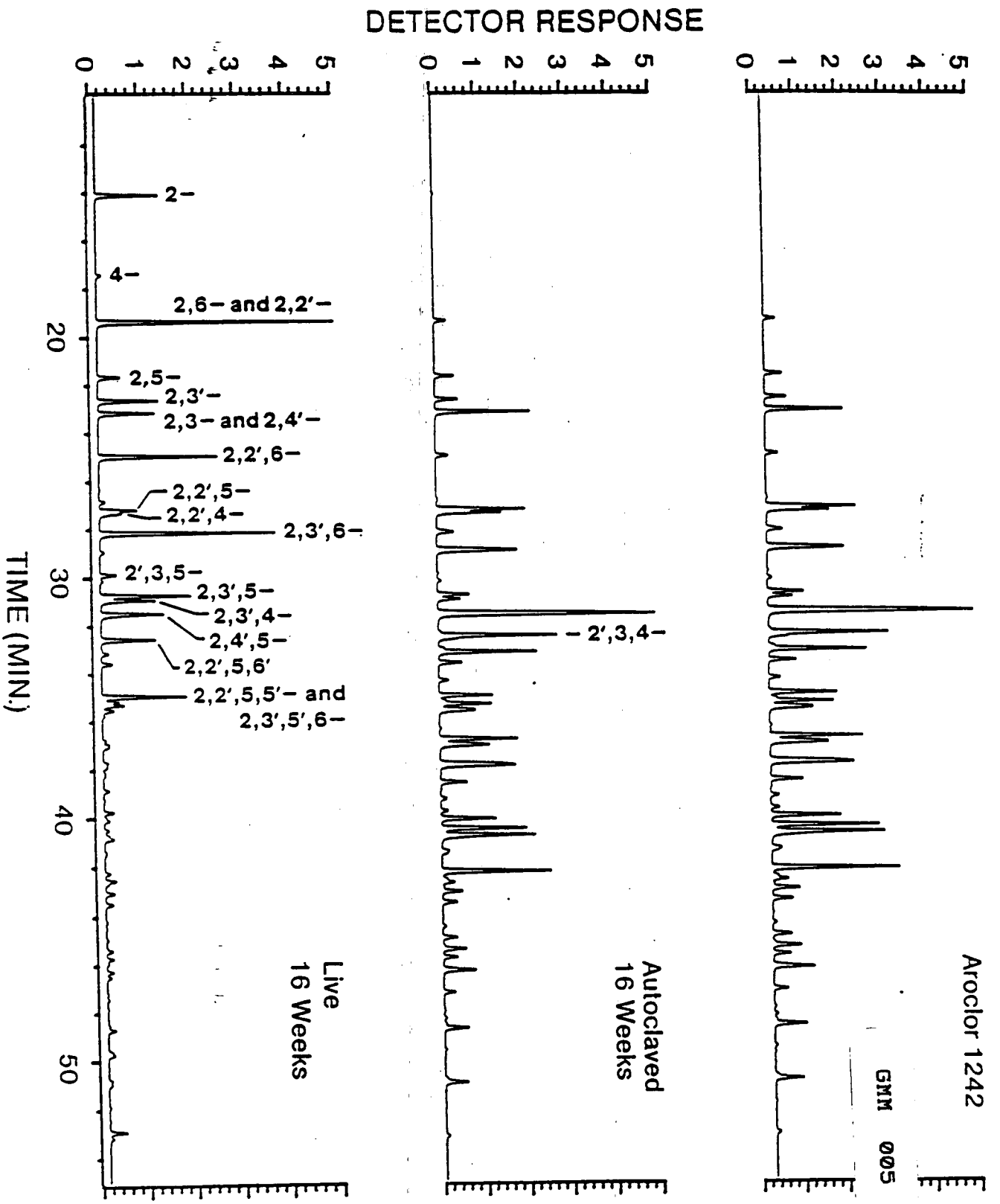
Figure 1: Capillary gas chromatograms showing the anaerobic dechlorination of Aroclor 1242 by Hudson River microorganisms. All chromatograms were normalized so that the highest peak had a height of 5. Peak identifications were based on matching retention times to standards (2-CB and 4-CB) or published relative retention indices (*17*). Quantitation of 2-CB and 4-CB was based on pure standards. Quantitation of other congeners was based on data on the composition of Aroclor 1242 provided by J. F. Brown, Jr. and R. E. Wagner. An electron capture detector was used.

Figure 2: Removal of chlorines by position at three Aroclor 1242 concentrations. Dechlorination was almost exclusively from the meta and para positions in the live treatments only. Zero time data for the 14 and 140 ppm treatments was not reliable because an insufficient amount of material was sampled, and it is not plotted.

Table 1

Congeners	Weeks 0	4	8	16
Mono-	0.0 ± 0.0	1.7 ± 0.5	50.1 ± 10.7	66.7 ± 3.4
Di-	9.1 ± 0.7	15.3 ± 0.2	25.5 ± 3.7	21.3 ± 0.6
Tri-	48.5 ± 0.1	48.2 ± 1.3	16.2 ± 4.9	8.5 ± 2.3
Tetra-	36.3 ± 0.6	30.0 ± 0.9	6.8 ± 1.8	3.0 ± 0.4
Penta-	5.2 ± 0.2	4.2 ± 0.2	1.3 ± 0.4	0.5 ± 0.1
Hexa-	0.9 ± 0.0	0.6 ± 0.0	0.2 ± 0.1	0.0 ± 0.0

Fig 1


DETECTOR RESPONSE
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4-
2,6- and 2,2'-
2,5-
2,3'-
2,3- and 2,4'-
2,2',6-
2,2',5-
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2,2',5,6'
2,2',5,5'- and
2,3',5',6-
20
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TIME (MIN.)
Live
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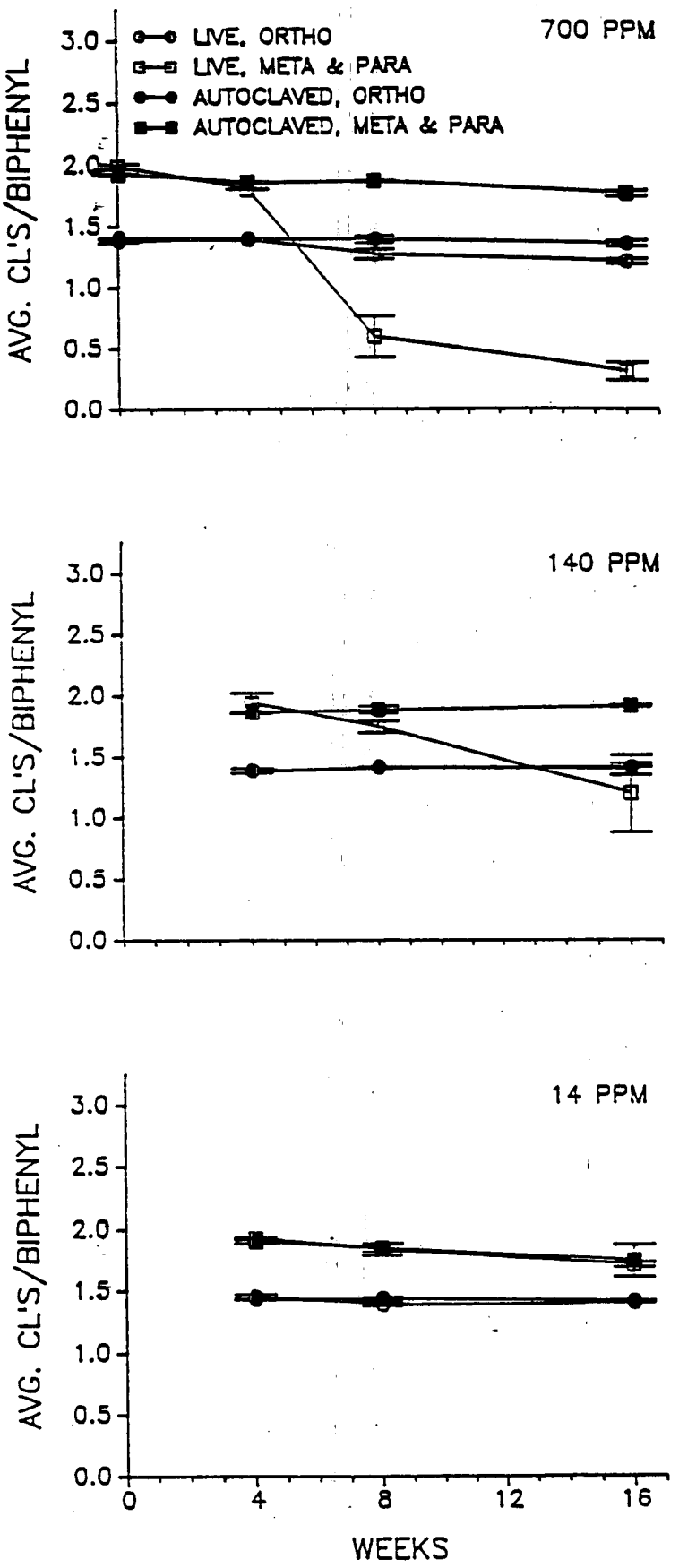
2',3,4-
Autoclaved
16 Weeks
Aroclor 1242
GMM 005 0208

Fig 2



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