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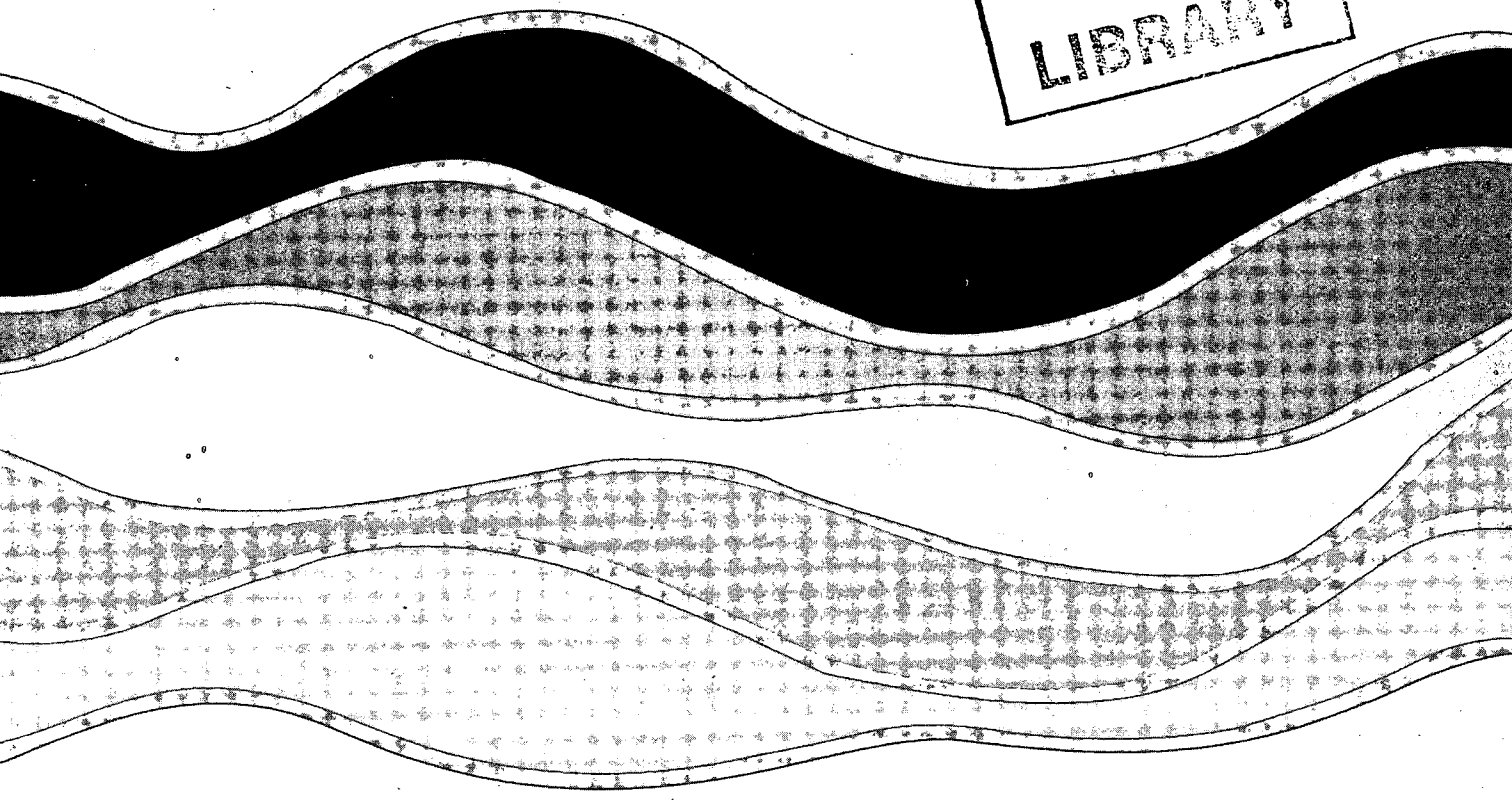

EFFECT OF SAMPLE PRESERVATION ON BODY BURDEN ANALYSIS OF ORGANIC CONTAMINANTS IN UNIONID MUSSELS

M. Hanna

NWRI Contribution No. 90-34

MANAGEMENT PERSPECTIVE

In earlier work, Metcalfe and Charlton (1989) found that the majority of PCB compounds entering the St. Lawrence River at Massena persisted in native mussels for several hundred kilometers downstream of the point source in a characteristic accumulation pattern. This was the rationale for our using native mussels from the St. Lawrence River, between Lake St. Louis and Tadoussac to concurrently assess and follow the evolution of contaminants (contaminant levels, sources, bioavailability and persistence). Unfortunately, the mussel samples collected in the summer of 1989 were subjected to temporary thawing out. Even though mussels are widely used as biomonitors, no studies have been carried out to actually quantify the effect of sample preservation. This is why an experiment to quantitatively assess the effect of sample preservation on the body burden analysis of organic contaminants in mussels was carried out.

PERSPECTIVE-GESTION

Metcalfe et Charlton (1989) ont constaté, lors de travaux antérieurs, que la plupart des PCB qui pénètrent dans le Saint-Laurent à Massena persistent dans les moules indigènes sur plusieurs centaines de kilomètres en aval de la source ponctuelle, en suivant un mode d'accumulation caractéristique. C'est cette constatation qui nous a incités à utiliser des moules indigènes vivant dans le Saint-Laurent entre le lac Saint-Louis et Tadoussac, pour évaluer et simultanément suivre l'évolution des contaminants (concentrations, sources, biodisponibilité et persistance). Malheureusement, les échantillons de moules prélevés au cours de l'été 1989 ont été temporairement décongelés. Les moules sont fréquemment utilisées comme bio-indicateurs, mais aucune étude n'a été effectuée pour mesurer l'effet de la préservation des échantillons. C'est pour cette raison que nous avons effectué une expérience destinée à évaluer quantitativement l'effet de la préservation des échantillons sur la détermination de la charge en contaminants des organismes.

ABSTRACT

In order to quantitatively assess the effect of the temporary thawing out of the St. Lawrence mussel samples, collected between Lake St. Louis and Tadoussac, a comparative analysis was carried out on the unionid mussel Elliptio complanata, resampled by Ponar dredge from one site in the Cornwall/Massena area. To adequately reproduce the conditions of the thawing out problem, the mussels were divided into two groups, while Group A was kept in the freezer at -20°C, Group B was kept in the refrigerator for five days at 5°C. All the compounds present in the control were also present in Group B samples. Analysis of the organic contaminants in each of these two groups showed that for total PCB concentrations, the two treatments were not significantly different; however, when compared individually 6 of the 13 congeners showed significant differences. The observed differences were relatively small for individual PCB congeners (7.1 to 15.3%), higher for chlorobenzenes (10.5 to 36.4%), and yet higher for HCE (44.1%); the difference for HCE, although large is nevertheless not significant, even if only marginally so.

RÉSUMÉ

On a effectué une analyse comparative de la moule Elliptio complanata (familles des unionidés) , qui avait été reprélevée à l'aide d'une drague Polar à un endroit dans la région de Cornwall/Massena, en vue d'évaluer quantitativement l'effet de la décongélation temporaire d'échantillons de moules du Saint-Laurent, prélevées entre le lac Saint-Louis et Tadoussac. Afin de reproduire convenablement les conditions associées au problème de la décongélation, nous avons divisé les moules en deux groupes, soit les groupes A et B. Les moules du groupe A ont été conservées au congélateur à -20 °C, tandis que les moules du groupe B ont été gardées au réfrigérateur pendant cinq jours à une température de 5 °C. Tous les composés présents dans le témoin se trouvaient également dans les échantillons du groupe B. Le dosage des contaminants organiques dans les moules de ces deux groupes a révélé que les deux traitements n'étaient guère différents pour ce qui est de la concentration des PCB totaux; toutefois, lorsqu'on les comparait individuellement, 6 des 13 congénères présentaient des différences importantes. Les

différences observées étaient relativement faibles pour les congénères individuels de PCB 7,1 à 15,3 %),plus élevées pour les chlorobenzènes (10,5 à 36,4 %) et plus élevées encore pour le HCE (44,1 %); la différence pour le HCE, bien qu'élevée, n'est toutefois pas importante, même marginalement.

EFFECT OF SAMPLE PRESERVATION ON BODY BURDEN ANALYSIS OF ORGANIC CONTAMINANTS IN UNIONID MUSSELS

INTRODUCTION

The purpose of this study was to quantitatively assess the effect of the temporary thawing out of the St. Lawrence mussel samples (collected between Lake St. Louis and Tadoussac, excluding those collected by J. Metclafe) on body burden analysis of organic contaminants and heavy metals.

MATERIALS AND METHODS

In order to quantitatively assess the effect of the temporary thawing out of unionid mussels, a comparative analysis of organic contaminants was carried out on the unionid mussel Elliptio complanata, resampled by Ponar dredge from one site in the Cornwall/Massena area on September 28, 1989. At the time of collection, the mussels were rinsed with river water to remove adhering sediment, then wrapped in pre-fired aluminum foil and frozen. After identifying the mussels collected, it was decided to use the 24 Elliptio complanata available because it is the dominant species in the area of interest in this study. These were divided into two groups of 10 mussels of equal lengths as measured with calipers (four mussels were empty).

To adequately reproduce the conditions of the thawing out problem, Group A was kept in the freezer -20°C while Group B was kept in the refrigerator for five days at 5°C. At the end of the five days, the mussels from Group B were returned to the freezer until they were shucked (while still frozen to avoid loss of body fluids) and placed in solvent-washed glass jars. The soft tissue from each group of mussels was weighed and thoroughly homogenized using a stainless steel blender. Subsequently, each homogenate was

ground with anhydrous Na_2SO_4 until the mixture was completely dry before the total weights were recorded. After complete grinding (glass pestle and mortar) and homogenization, each of these samples was subsampled three times, the weight of each subsample being recorded.

Each of the six subsamples (three replicates for each of the two treatments) was soxhlet-extracted and cleaned-up as described in the ANALYTICAL METHODS MANUAL (1990, in press) and analyzed by dual capillary column gas chromatography for 13 PCB congeners and 34 organochlorines (Tables 1 and 2) using dual electron capture detectors (Fox and Carey 1989).

RESULTS AND DISCUSSION

POLYCHLORINATED BIPHENYLS

Table 1 summarizes the comparison between the two treatments; it contains the concentrations of each polychlorinated biphenyl (PCB) congener for the two treatments, A (control) and B (treatment), as well as the statistical comparison. Total PCB concentrations were examined prior to individual congeners. In order to statistically compare the results obtained by the two treatments, the absolute concentrations of PCBs were logarithmically transformed before computing the variances for each treatment and for each congener.

All the congeners that were detectable in the control were also detectable in the treatment, but at lower concentrations. Congeners #18, 149 and 118 gave unreliable analytical values due to interference by other compounds; this was the case for the two treatments. These compounds were therefore eliminated from the statistical analysis.

The control group showed higher variances and therefore less precision than the thawed out samples for most congeners tested except for congeners #44 (tetra), 101 and 105 (penta). To determine whether or not the two treatments are significantly different, the sum of the ratio VAR A_1/VAR B_1 was computed, for each congener. Comparison of the obtained value with the F-distribution (2 degrees of freedom, $F_{0.95} = 19$) indicates that the two treatments are not significantly different, as far as total PCBs are concerned. However, further testing is necessary to compare the individual congeners, therefore computing:

$$(\text{VAR } A_1 + \text{VAR } B_1)/2 = \alpha_1^2 \qquad (1)$$

$$(\text{MEAN } A_1 - \text{MEAN } B_1)/\alpha_1 * (2/3)^{0.5} \qquad (2)$$

and, the t-distribution (4 degrees of freedom, $t^{0.95} = 2.13$), revealed that the two treatments are significantly different for six of the 13 congeners: #44, 49, 52 (tetra), 101 (penta), 151 (hexa) and 183 (hepta) (Table 1). The following congeners: #105 (penta), 138 (hexa), 180 (hepta), 194 (octa), did not show a significant difference between the two treatments.

Figure 1 illustrates the differences in the concentrations of each of the congeners tested. Figure 2 illustrates the variance between each control replicate and the average control concentration for each congener. Figure 3 illustrates the variance between each treatment replicate and the average control concentration, the 1:1 line representing equivalence between the treatment and the average control.

The difference between the two treatments was quantified by using the ratio:

$$((\text{MEAN } A_1 - \text{MEAN } B_1)/\text{MEAN } A_1) * 100 \qquad (3)$$

the concentrations obtained for the treatment ranged from 7.1 (Congener 194) to 15.3% (Congener 44), lower than the concentrations obtained from the control (Table 1).

Of the 13 PCB congeners analyzed, 12 have been recommended among the priority polychlorinated biphenyl congeners for congener-specific analysis offered for use in the regulatory evaluation of dredged material (McFarland and Clarke 1989).

- Two congeners, #118 and 138, are classified as most likely to contribute adverse biological effects attributable to PCBs in an environmental sample. They are mixed-type inducers, reported frequently in environmental samples. These two congeners did not show a significant difference between the treatment and the control.

- Four congeners, #101, 180, 183 and 194, are PB-type inducers prevalent in the environment. Two of these (180 and 194) did not show a significant difference between the two treatments.

- Five congeners, #18, 44, 49, 52 and 151, are weak or non-inducers, but they occur either frequently in the environment or in high concentrations in animal tissues relative to other PCB congeners. While #18 was not detectable in our samples, the other four presented significantly lower concentrations for thawed out samples.

- One congener, #105, is a mixed-type inducer, reported infrequently and in very low tissue concentrations in biota. No significant difference between the two treatments, was observed for this congener.

OTHER ORGANOCHLORINES

Table 2 summarizes the comparison of the responses of the organochlorine (OC) analysis obtained by the two treatments; it contains the concentrations of each compound for the two treatments, A (control) and B (treatment), as well as the

statistical comparison. The two treatments were compared in the same manner as were PCBs.

All the compounds that were detectable in the control were also detectable in the treatment, but at lower concentrations, these are:

- HCE (a substituted linear hydrocarbon)
- 1,2,4 TCB
- 1,2,3,5 TeCB
- 1,2,3,4 TeCB
- PeCB showed reliable values for the control only
- 1,2 DCB showed reliable values for the treatment only.

The following compounds yielded undetectable concentrations for the two treatments:

- 2,3,4,6 TeCA
- PeCA
- lindane
- heptachlor
- OCS
- ϑchlordane
- trans nonachlor
- dieldrin.

The values obtained for the remaining compounds (1,3 DCB, 1,4 DCB, 1,3,5 TCB, 1,2,3 TCB, 1,2,4,5 TeCB, αBHC, HCB, aldrin, o,p,DDE, αendosulfan, αchlordane, p,p' DDE, o,p DDD, endrin, βendosulfan, p,p' DDD, o,p DDT, p,p' DDT, methoxychlor and mirex) proved to be unreliable for the two treatments, due to interference by other compounds, their concentrations were therefore non quantifiable. To guarantee a more conservative statistical analysis, all the non detectable (ND) and non quantifiable (NQ) compounds were eliminated from the analysis. No conclusion should be drawn with regards to these two groups of compounds.

The control group showed lower variances and therefore more precision than the thawed out samples for all the compounds

detected. Testing of each compound individually indicates that for HCE
1,2,3,5 TeCB
1,2,3,4 TeCB
PeCB
the two treatments gave non significantly different responses, whereas they showed a significant difference for: 1,2 DCB (which was only detected in Group B samples) and 1,2,4 TCB (Table 2).

Figure 4 illustrates the differences in the concentrations of each of the compounds tested. Figure 5 illustrates the variance between each control replicate and the average control concentration for each compound. Figure 6 illustrates the variance between each treatment replicate and the average control concentration, the 1:1 line representing equivalence between the treatment and the average control.

The difference between the concentrations obtained from the two treatments ranged from 10.5 to 36.4% for chlorobenzenes and 44.1% for HCE, a substituted linear hydrocarbon that is more amenable to biological degradation (Table 2). This large difference for HCE is nevertheless non significant (marginally so), according to equation 2.

HEAVY METALS

No experimental work was carried out for heavy metals because there seems to be a concesus (B. Bourgoin, G. Jamro, K. Lum, S. Luoma, A. Mudroch, pers. comm.) on the fact that because of their stable and persistent nature, total metals would not be affected in any way by the thawing out. There might be an effect on metal speciation, however this would not affect this study since during the analysis for total metals all forms of the metal would show up in the total value.

Dr. Sam Luoma (pers. comm.) has worked extensively on heavy metals in marine invertebrates, and thinks that it is unlikely that any effect would be observed for heavy metals, as a consequence of the thawing out. Dr. Luoma has experienced a similar problem with crabs and observed no effect on total metal concentration, provided the work is carried out on whole body analysis, as is the case in our study, and not on particular organs (S. Luoma, pers. comm.). He observed some heavy metal migrations from one organ to the other during the thawing out, and if the study had been restricted to specific organs then there would have been a difference in metal distribution in specific organs.

Even for mercury, Dr. Luoma believes that the difference would be insignificant because most of the mercury found in invertebrates is mostly inorganic mercury and therefore these organisms contain little if any of the volatile methyl-mercury (S. Luomo, pers. comm.). If this is the case, then we can confidently assume that no significant effect will be observed even for mercury. This needs to be verified for freshwater mussels.

CONCLUSIONS

For total PCB concentrations, the two treatments are not significantly different (p=5%). For PCBs, the thawing out treatment gave smaller variances and therefore more precision in the response than did the control, whereas for the other organochlorines the contrary was true. All of the PCB congeners and other organochlorines that were detected in the control were also detected in the treatment, but in lower concentrations. The two congeners assigned as most likely to contribute to adverse biological effects did not show significant differences between the treatment and the control. The thawing treatment gave significantly different responses ranging from 10.1 to 15.3% for

congeners #44, 49, 52, 101, 151 and 183 (tetra, penta, hexa and hepta). Significant differences were also observed for the following chlorobenzenes: 1,2 DCB (only reliable for the treatment) and 1,2,4 TCB (36.4%). These differences are relatively small for PCBs (7.1-15.3%), higher for chlorobenzenes (10.5-36.4%), and yet higher for HCE (44.1%); the difference for HCE although large is nevertheless non significant, even if only marginally so. These results confirm the decreasing chemical and biochemical stabilities of these classes of compounds, from the most stable high molecular weight aromatic rings of PCBs to the least stable, lower molecular weight, linear configuration of HCE. No conclusion can be drawn for the compounds that were either non detectable or non quantifiable in our samples.

The stable and persistent nature of heavy metals would indicate that they would not be affected by the thawing out. Ranking the groups of compounds according to the extent of the effect of the treatment on the analytical responses, yields heavy metals as the least affected, followed by PCBs and chlorobenzenes to the most affected linear hydrocarbon HCE.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1. Concentrations of the studied Polychlorinated biphenyl congeners in mussel tissue from Treatment A (■) and Treatment B (+).

Figure 2. Relationship between the PCB congener concentrations for Treatment A (control) replicates and the average control concentrations.

Figure 3. Relationship between the PCB congener concentrations for Treatment B replicates and the average control concentrations.

Figure 4. Concentrations of the studied organochlorines in mussel tissue from Treatment A (■) and Treatment B (+).

Figure 5. Relationship between the organochlorine concentrations for Treatment A (control) replicates and the average control concentrations.

Figure 6. Relationship between the organochlorine concentrations for Treatment B replicates and the average control concentrations.

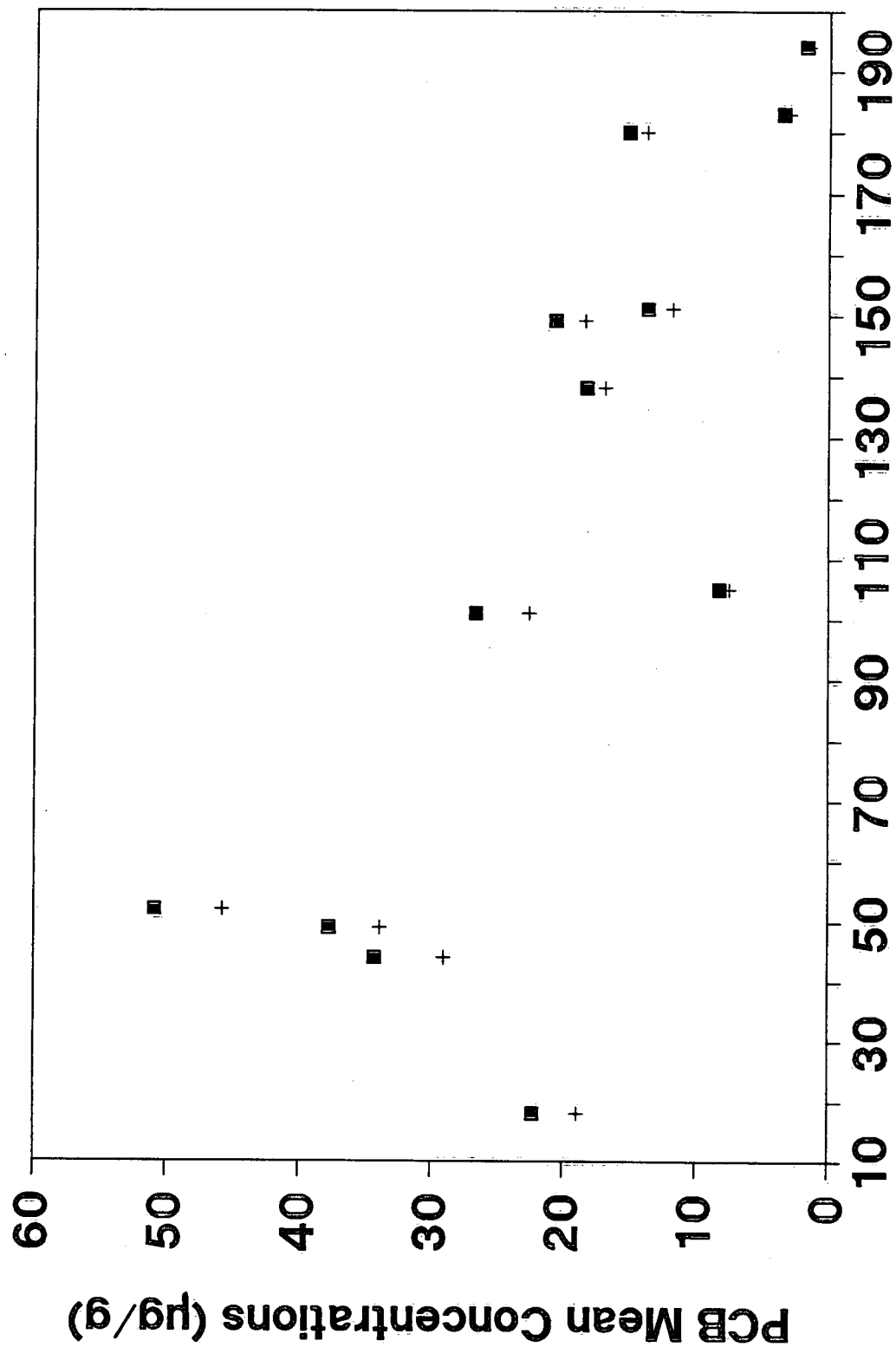

PCB Mean Concentrations (µg/g)
0
10
20
30
40
50
60
10
30
50
70
90
110
130
150
170
190
PCB Congener
Treatment A
Treatment B

FIGURE 1.

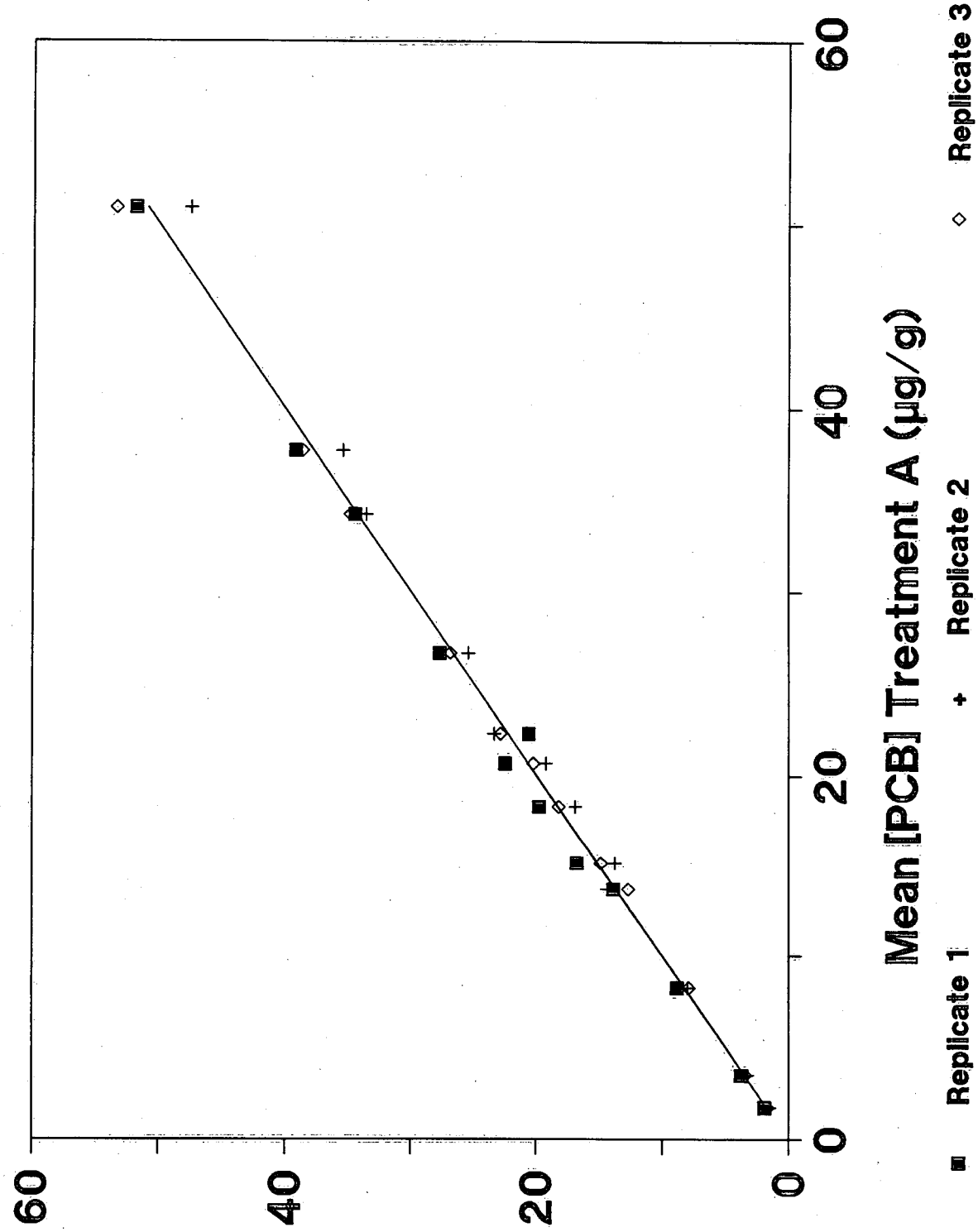

[PCB] Treatment A replicates (µg/g)
0
20
40
60
Mean [PCB] Treatment A (µg/g)
0
20
40
60
Replicate 1
Replicate 2
Replicate 3

FIGURE 2.

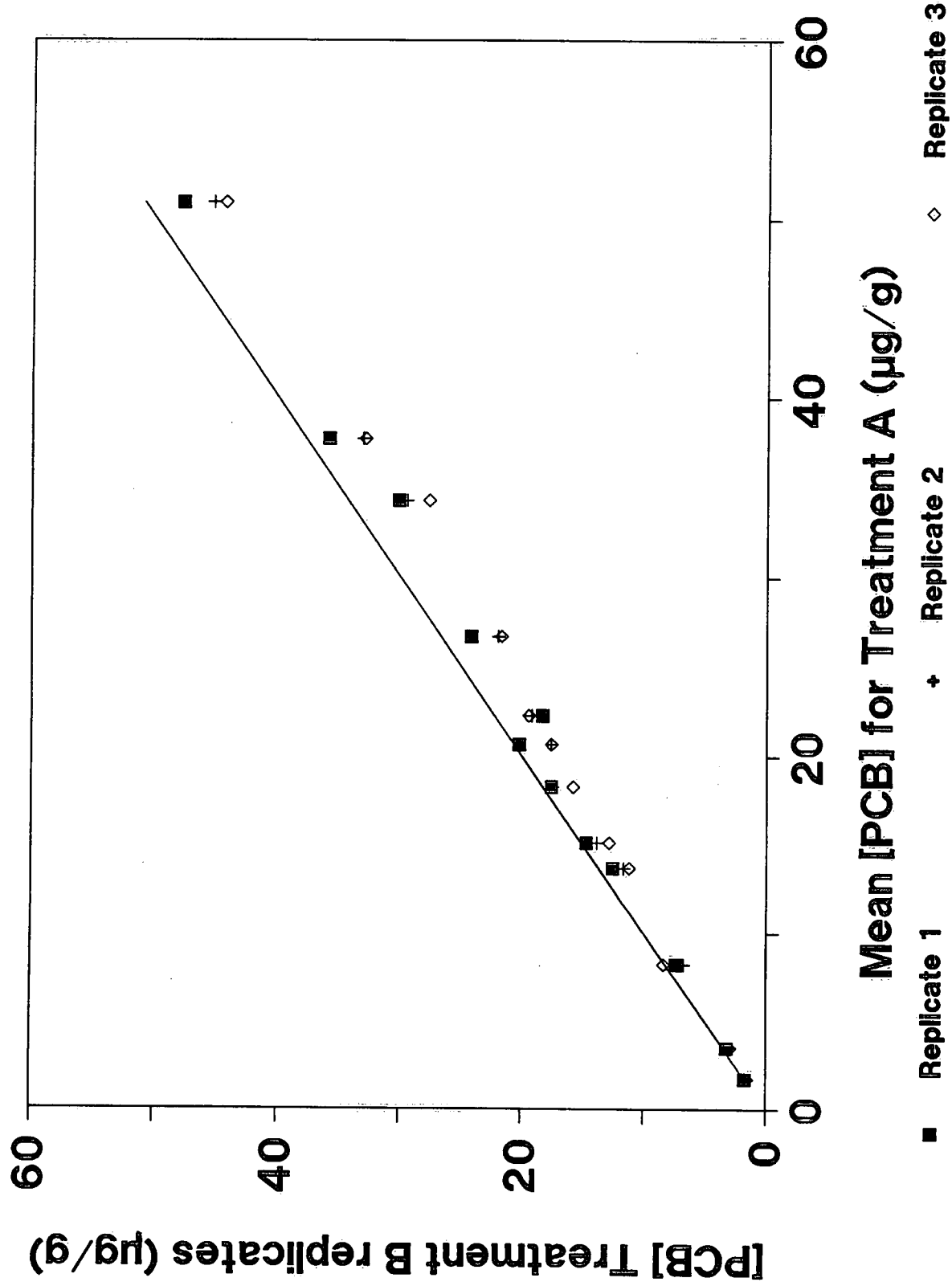

[PCB] Treatment B replicates (µg/g)
0
20
40
60
Mean [PCB] for Treatment A (µg/g)
0
20
40
60
Replicate 1
Replicate 2
Replicate 3

FIGURE 3.

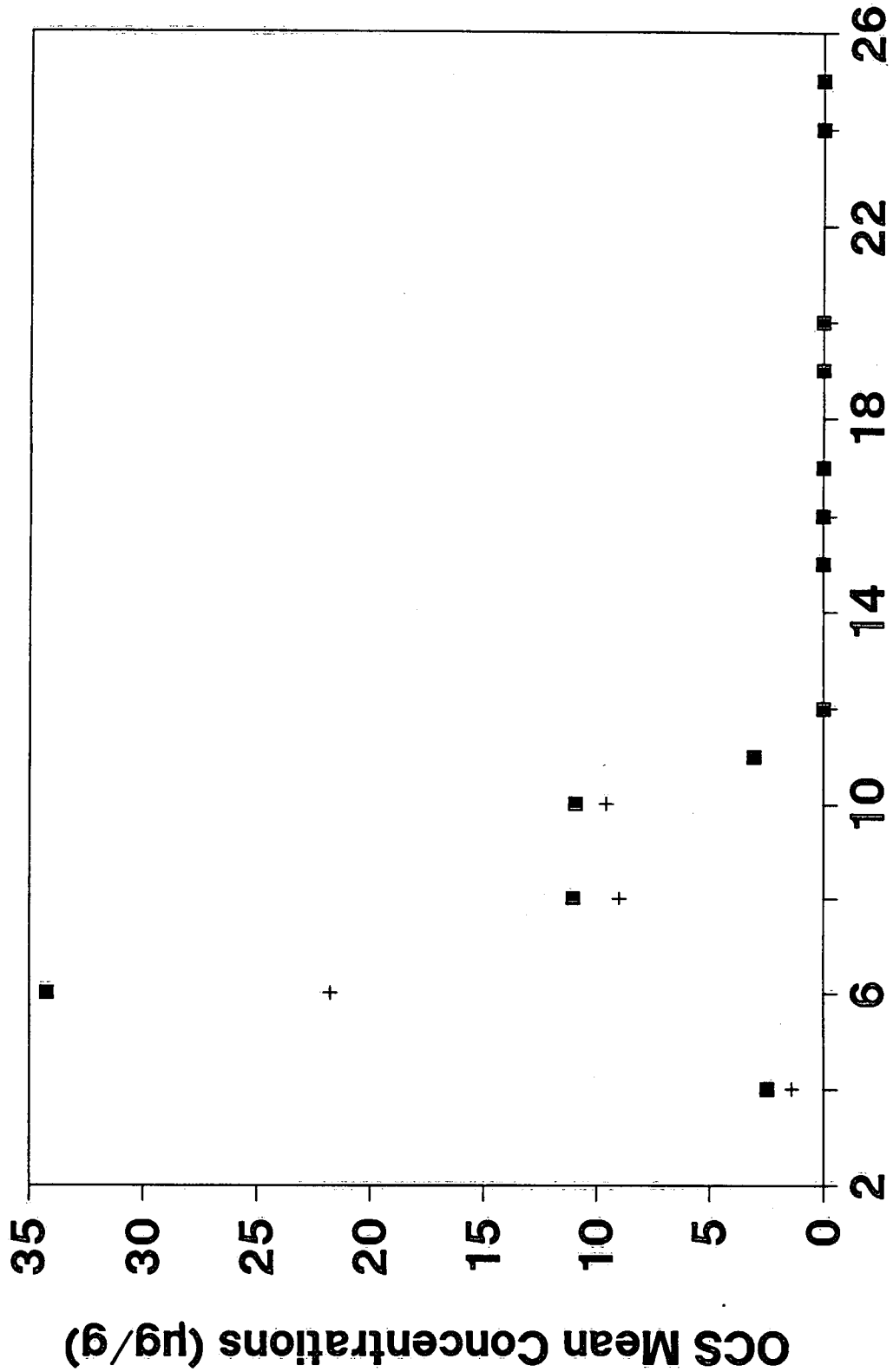

OCS Mean Concentrations (µg/g)
0
5
10
15
20
25
30
35
2
6
10
14
18
22
26
Organochlorines
Treatment A
Treatment B

FIGURE 4.

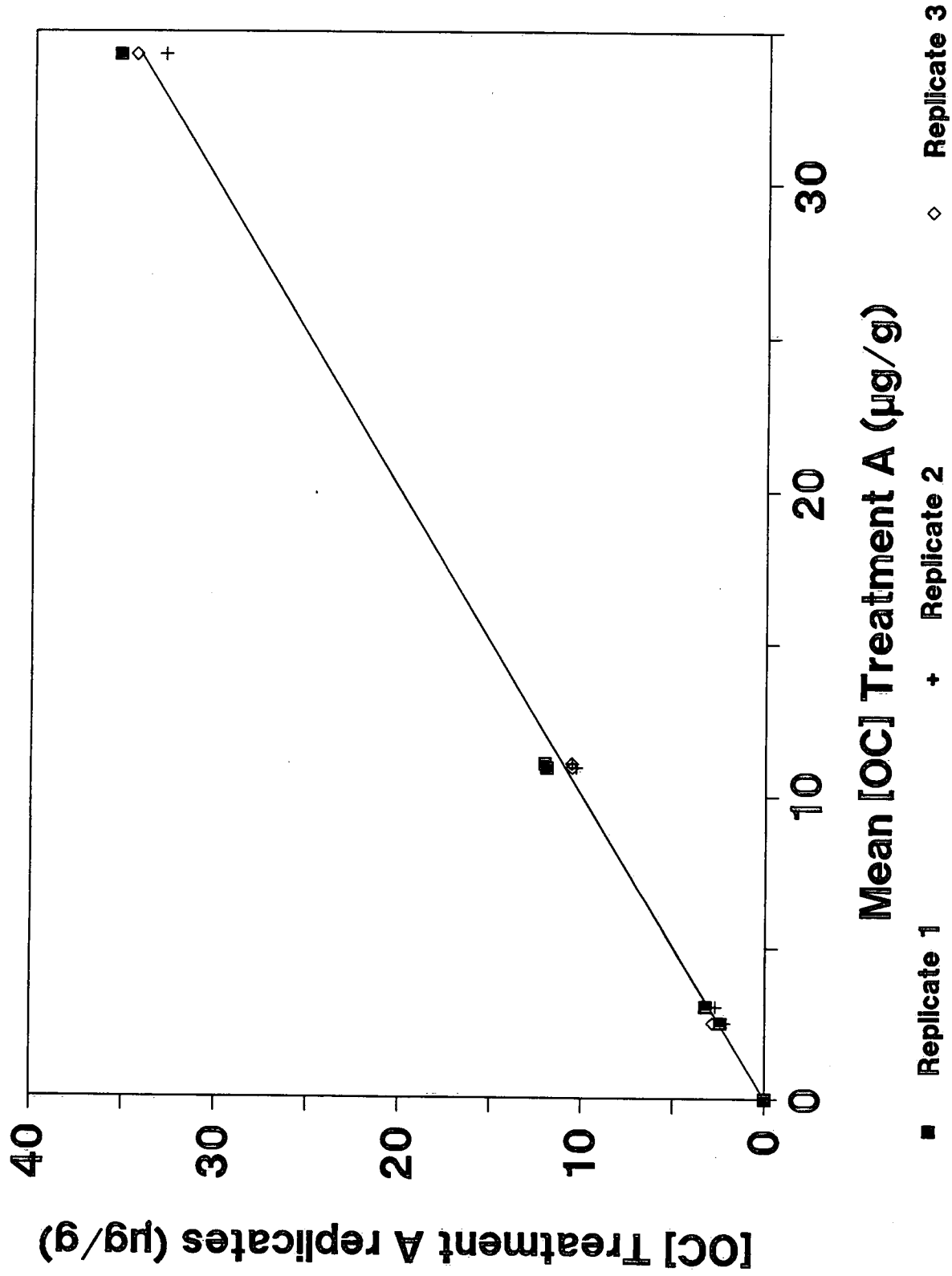

[OC] Treatment A replicates (µg/g)
0
10
20
30
40
Mean [OC] Treatment A (µg/g)
0
10
20
30
Replicate 1
Replicate 2
Replicate 3

FIGURE 5.

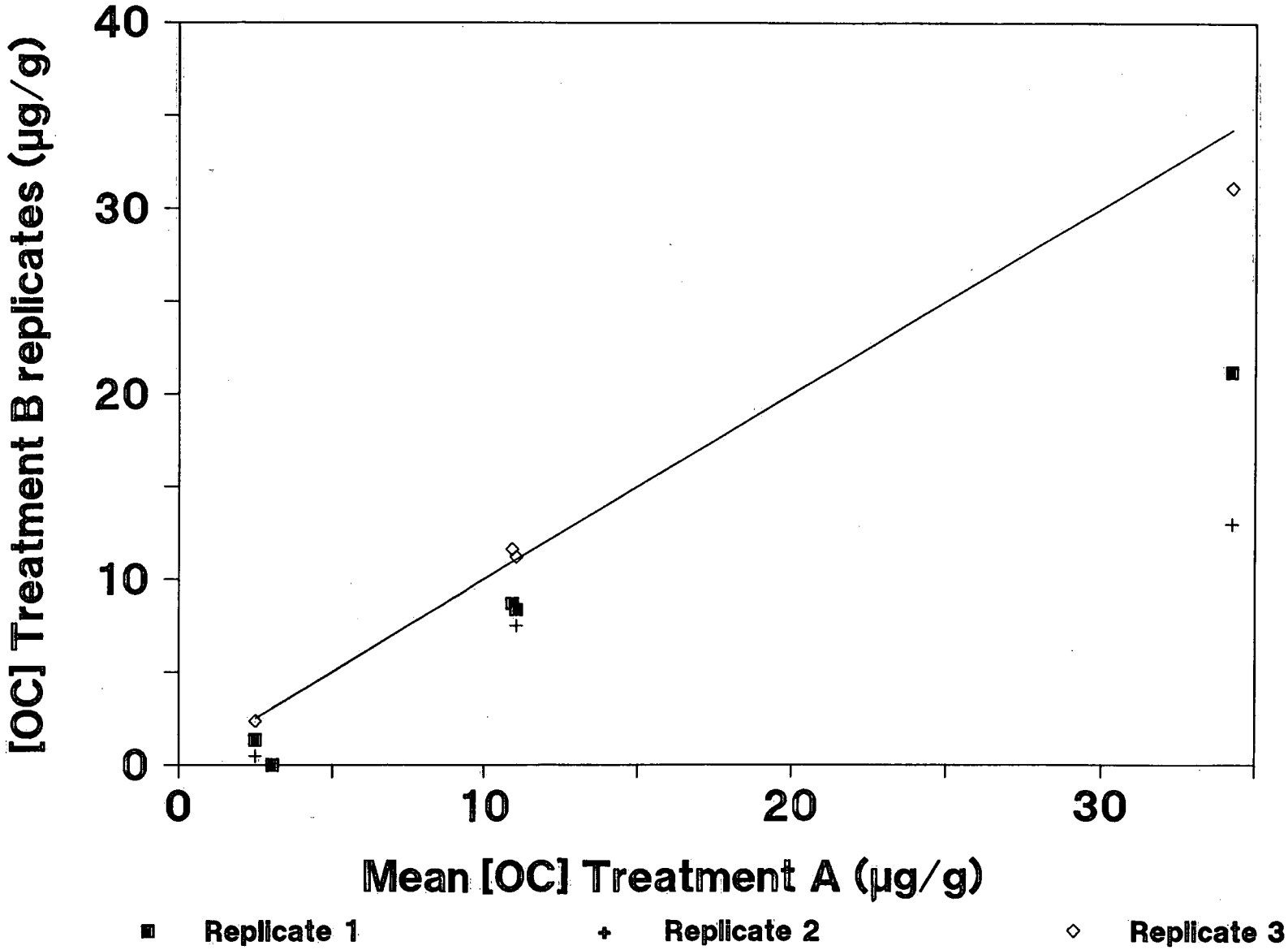

40
30
20
10
0
[OC] Treatment B replicates (µg/g)
0
10
20
30
Mean [OC] Treatment A (µg/g)
Replicate 1
Replicate 2
Replicate 3

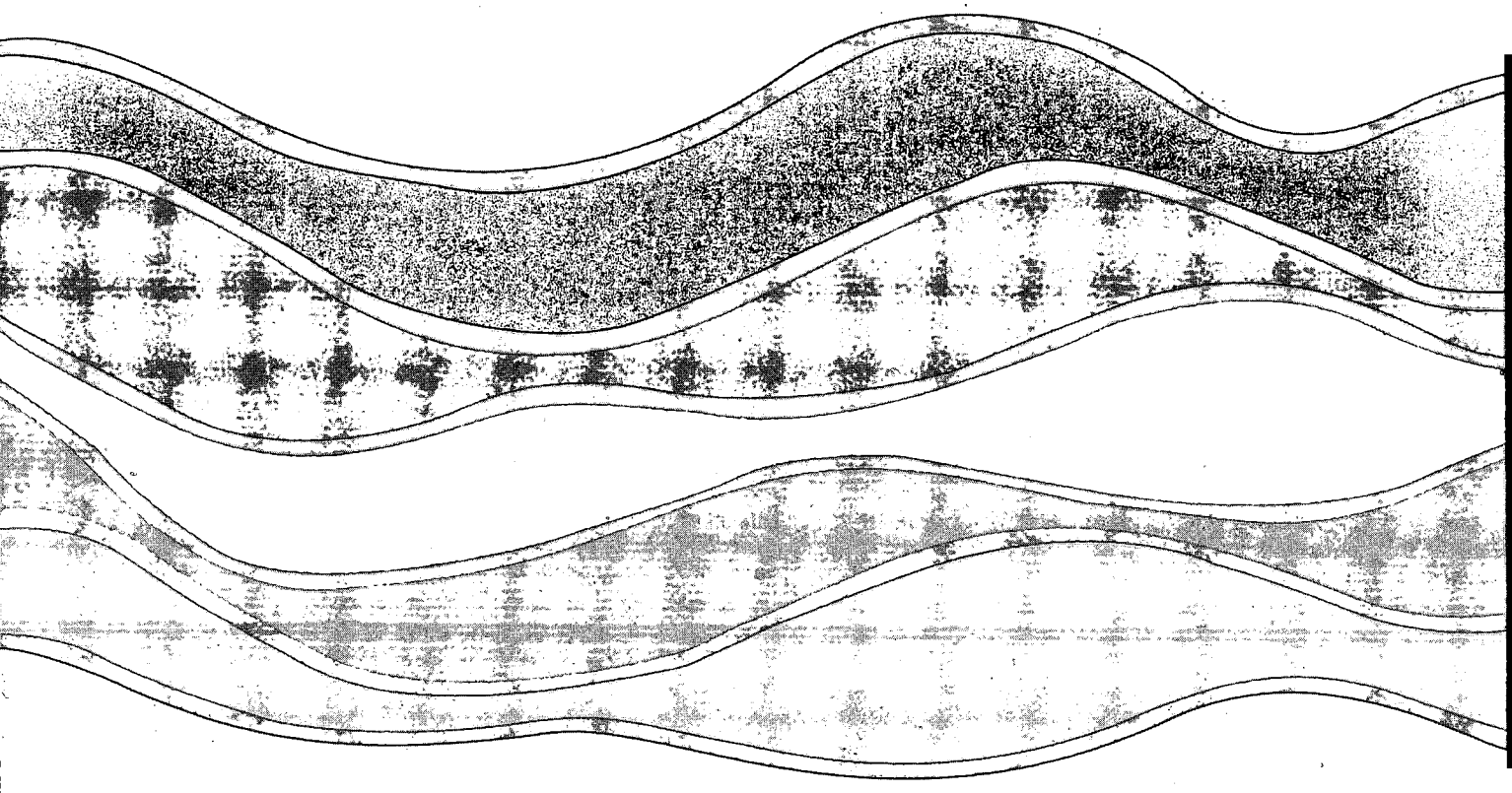
FIGURE 6.

TABLE 1. Comparison of the responses of 13 PCB congeners obtained by the control (A) and the treatment (B). The results are reported on a wet weight basis. NQ = non quantifiable.

PCB Congener	SAMPLE 1A µg/g	SAMPLE 2A µg/g	SAMPLE 3A µg/g	SAMPLE 1B µg/g	SAMPLE 2B µg/g	SAMPLE 3B µg/g	VARIANCE	% DIFF.
18	NQ	NQ	NQ	NQ	NQ	NQ	---	---
44	34.46	33.56	34.82	30.12	29.43	27.59	-7.17	-15.26
49	39.17	35.38	38.63	35.85	33.04	32.83	-3.07	-10.13
52	51.83	47.48	53.39	47.76	45.27	44.30	-3.09	-10.07
101	27.72	25.41	26.85	24.16	21.96	21.68	-4.77	-15.23
105	8.81	7.95	7.89	7.36	6.70	8.34	-1.65	-9.10
138	19.77	16.90	18.19	17.56	17.47	15.76	-1.63	-7.43
151	13.90	14.41	12.71	12.54	11.63	11.19	-3.63	-13.79
180	16.78	13.76	14.86	14.69	13.82	12.81	-1.62	-9.00
183	3.74	3.22	3.43	3.19	2.98	2.93	-3.16	-12.39
194	1.92	1.55	1.66	1.72	1.52	1.52	-1.14	-7.14
149+118	NQ	NQ	NQ	NQ	NQ	NQ	---	---

TABLE 2. Comparison of the responses of 34 organochlorines obtained by the control (A) and the treatment (B). The results are reported on a wet weight basis. ND = non detectable and NQ = non quatifiable.

CODE	OC Compound	SAMPLE 1A µg/g	SAMPLE 2A µg/g	SAMPLE 3A µg/g	SAMPLE 1B µg/g	SAMPLE 2B µg/g	SAMPLE 3B µg/g	VARIANCE	% DIFF.
1	1,3 DCB	NQ	NQ	NQ	NQ	NQ	NQ	---	---
2	1,4 DCB	NQ	NQ	NQ	NQ	NQ	NQ	---	---
3	1,2 DCB	NQ	NQ	NQ	63.22	18.49	108.66	9.16	---
4	HCE	2.43	2.20	2.85	1.34	0.48	2.36	-2.00	-44.06
5	1,3,5 TCB	NQ	NQ	NQ	NQ	NQ	NQ	---	---
6	1,2,4 TCB	35.34	32.91	34.47	21.19	12.98	31.12	-2.48	-36.44
7	1,2,3 TCB	NQ	NQ	NQ	NQ	NQ	NQ	---	---
8	1,2,3,5 TeCB	12.03	10.55	10.57	8.35	7.49	11.18	-2.09	-18.49
9	1,2,4,5 TeCB	NQ	NQ	NQ	NQ	NQ	NQ	---	---
10	1,2,3,4 TeCB	11.90	10.31	10.55	8.67	8.41	11.62	-1.54	-12.42
11	PeCB	3.25	2.69	3.20	NQ	NQ	NQ	-1.48	-10.51
12	2,3,4,6 TeCA	0.00	0.00	0.00	0.00	0.00	0.00	---	---
13	a BHC	NQ	NQ	NQ	NQ	NQ	NQ	---	---
14	HCB	NQ	NQ	NQ	NQ	NQ	NQ	---	---
15	PeCA	ND	ND	ND	ND	ND	ND	---	---
16	LINDANE	ND	ND	ND	ND	ND	ND	---	---
17	HEPTACHLOR	ND	ND	ND	ND	ND	ND	---	---
18	ALDRIN	NQ	NQ	NQ	NQ	NQ	NQ	---	---
19	OCS	ND	ND	ND	ND	ND	ND	---	---
20	g CHLORDANE	ND	ND	ND	ND	ND	ND	---	---
21	o,p DDE	NQ	NQ	NQ	NQ	NQ	NQ	---	---
22	a ENDOSULFAN	NQ	NQ	NQ	NQ	NQ	NQ	---	---
23	a CHLORDANE	NQ	NQ	NQ	NQ	NQ	NQ	---	---
24	t NONACHLOR	ND	ND	ND	ND	ND	ND	---	---
25	DIELDRIN	ND	ND	ND	ND	ND	ND	---	---
26	p,p' DDE	NQ	NQ	NQ	NQ	NQ	NQ	---	---
27	o,p DDD	NQ	NQ	NQ	NQ	NQ	NQ	---	---
28	ENDRIN	NQ	NQ	NQ	NQ	NQ	NQ	---	---
29	b ENDOSULFAN	NQ	NQ	NQ	NQ	NQ	NQ	---	---
30	p,p' DDD	NQ	NQ	NQ	NQ	NQ	NQ	---	---
31	o,p DDT	NQ	NQ	NQ	NQ	NQ	NQ	---	---
32	p,p' DDT	NQ	NQ	NQ	NQ	NQ	NQ	---	---
33	METHOXYCHLOR	NQ	NQ	NQ	NQ	NQ	NQ	---	---
34	MIREX	NQ	NQ	NQ	NQ	NQ	NQ	---	---



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