CONGENER-SPECIFIC ANALYSIS AND TOXIC POTENTIAL OF POLYCHLORINATED BIPHENYLS IN SNAPPING TURTLE EGGS FROM OHIO LAKE ERIE BASIN

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INTODUCTION

Characteristics of the snapping turtle's life style and their ubiquitous distribution throughout the United States and Canada make them a valuable bioindicator of chemical exposure and the effects of environmental contaminants. The species lives within relatively small areas (approximately 3-4 ha) and usually remains in the same home range in consecutive years (Obbord and Brooks, 1981). They are omnivorous and have the potential to accumulate high levels of lipophilic organochlorine contaminants (OCs) through their diet.

A considerable amount of information on OCs including polychlorinated biphenyl (PCB) levels in snapping turtles is available for specimens inhabiting the Canadian part of the Great Lakes Basin. No research in this respect has been conducted on specimens living in the Lake Erie Basin in Ohio. There is lack of information regarding contaminant profiles and levels in snapping turtles in Ohio waters.

PCBs continue to be of concern due to their persistence and potential to produce adverse health effects in living organisms. Bioaccumulation and toxicity of PCBs is structure dependent. Laboratory *in vitro* and *in vivo* experiments have shown that the non-ortho chlorinated dioxin-like PCBs, IUPAC numbers 77, 126 and 169, are the most toxic and cause similar effects in mammals, birds and fish. The spectrum of toxic effects includes body weight loss, dermal disorders, thymic atrophy, teratogenicity, immunotoxicity and reproductive toxicity and induction of CYP1A gene expression (Safe 1994). Although there is considerable variation in species sensitivity, developing embryos are more sensitive than adult organisms. Mono-ortho-chlorinated PCBs produce toxic responses as well, but are less active in elucidating dioxin-like activity (Walker and Peterson, 1981).

The objective of this study was to provide information about total and congener-specific levels of PCBs and to determine the toxicological significance of non-ortho, mono-ortho and di-ortho chlorinated congeners encountered in the turtle egg samples.

METHODS

Collection of turtles

Adult female snapping turtles (*Chelydra serpentina*) were collected in June and July of 1997 from 6 sites in the Lake Erie Basin in Ohio. The sites were: Ottawa River in Toledo (OR), river mile-4.50; Maumee River mouth (MR), river mile-1.0; Black River (BR), river mile-1.98; Ashtabula River (AR), river mile-1.60; Ottawa National Wildlife Refuge (ONWR) and Lake Rockwell (LR), river mile-61.40. The sites varied in contamination levels and contaminant spectrum. ONWR and LR were considered uncontaminated and served as a reference sites. ONWR is a marsh located on the Lake Erie shore, while LR is a drinking water reservoir for the City of Akron.

Turtles were captured using baited traps and transported to the Ohio State University laboratory. The turtles received deep anesthesia in the laboratory after which they were decapitated and dissected. Eggs were removed, placed on aluminum foil, counted, weighed and frozen at –20°C pending analysis.

Chemical Analysis

The content of 7-8 whole eggs from each female were selected at random, pooled, homogenized and prepared for analysis using a procedure similar to described by Bishop et al (1996). Briefly, a subsample of egg homogenate (5g) was dehydrated by grinding with an excess of anhydrous sodium sulfate (6:1) and extracted on a chromatographic column with dichloromethane (DCM): hexane (1:1 v/v). Before extraction PCBs IUPAC numbers 14, 65 and 166 were added to all samples and blanks as surrogate standards. The extract was concentrated in a rotoevaporator. For clean-up, Florisil and silica columns were used with hexane as the eluant. The final extracts were solvent-exchanged to isooctane and concentrated . Internal standards, IUPAC congeners #30 and #204, were added and the volume was adjusted to 1 or 2 ml.

The identification and quantification of PCB congeners was performed using a Hewlett-Packard 5890 gas chromatograph (GC) equipped with a Ni^{63} electron capture detector (ECD), a fused silica capillary column (60 m long, 0.25 mm i.d., 0.25um film thickness, J&W DB5), autosampler and a Hewlett-Packard Chemstation for data acquisition. The GC operating conditions were: splitless injection; injection volume, 1ul; carrier gas, H_2; make-up gas, N_2; injection temp, 250°C; detector temp, 325°C; initial temp,100°C; first ramp rate, 1°C/min to 265°C; second ramp rate, 20°C/min to 300°C; final hold, 0 min. Each chromatographic peak was quantified by the internal standard method. The calibration standard was a mixture of Aroclors 1232, 1248 and 1262 as described by Mullin (1984).

Concentrations of non-ortho PCBs were measured in an aliquot of the hexane extract prepared for analysis according to the procedure described by Lazar et al (1992) with a few modifications. Briefly, to separate the non-ortho PCBs from other congeners a glass chromatographic column was packed with 5% carbon/silica gel between two layers of anhydrous sodium sulfate. Extracts were sequentially eluted with hexane (Fr1) and on inverted column with toluene (Fr2). The Fr2 fraction contained the non-ortho congeners. The fraction was concentrated, solvent-exchanged to isooctane and final volume was brought to 0.4 mL.

Lipid content was determined gravimetrically in a subsample of chloroform:methanol (2:1 v/v) extracted egg homogenate (Folch et al.,1957).

Toxic equivalents (TEQs) were determined from concentrations of individual PCB congeners in an additive model, i.e.: TEQ = Ó[$(PCB)_i$ * $(TEF)_i$], i =individual PCB congener concentration, TEF = toxic equivalency factor. The TEQs were calculated using the TEFs proposed to WHO-ECEH by Ahlborg et al (1994). The included congeners were non-ortho-chlorinated numbers 77, 81, 126 and 169, as well as some mono-and di-chlorinated congeners, i.e., numbers 118, 156, 167, 189 and 180.

Statistical analysis

Total PCB levels, lipid content and concentrations of non-ortho-chlorobiphenyls were compared among locations with a one-way analysis of variance (ANOVA). PCB concentrations were log-transformed to stabilize variances. Significant differences were determined using Tukey's multiple comparisons procedure.

QA/QC

1. Recoveries of individual PCB congeners were determined in matrix spikes containing Mullin's Aroclor mix carried through the analytical procedure. Mean recovery was 108%, with a SD of 17%. Mediana was 106%; 92% of the congeners had recoveries in the range of 80-130%.
2. Three surrogate standards, PCB congeners IUPAC numbers 14, 65 and 166, were added prior to extraction to all samples and blanks to monitor the analytical efficiency and accuracy. The recoveries for these congeners were (%, n=18, mean±SD): PCB 14, 92±8; PCB 65, 98±13; PCB 166, 106±22. Reported PCB concentrations were not recovery-corrected.
3. Procedural blanks were performed with each set of 8 samples. Blank responses were generally less than respective MDLs.
4. Precision was determined by measurement of individual PCB congeners in duplicate in samples taken randomly from two locations, and has been expressed as relative percent difference (RPD). The RPDs were less than 30% for 87% of the PCB peaks and were greater than 50% for 6% of PCB peaks.

RESULTS

Total PCB concentrations and PCB homologues profile

Turtle weight, mean egg weight and egg lipid content are given in Table 1. There was no correlation between lipid content and total PCB concentrations in the eggs. Therefore the PCBs were not lipid normalized.

Total PCB and PCB homologue concentrations are reported in Table 2 as the sum concentration of individual PCB congeners. Total PCBs were significantly different between contaminated and reference locations. However, no significant difference has been found among contaminated sites due to low sample size. The greatest total PCB concentations were determined in OR and MR eggs, 3683 ng/g and 2519 ng/g wet weight, respectively. Reference sites ONWR and LR had total PCBs of 352 ng/g and 183 ng/g , respectively.

The PCB homologue profile revealed that at all collection sites except for OR, hexa-chlorobiphenyls (hexa-CBs) were dominant followed by hepta- and penta- congeners. The relative proportions of hexa-CBs in total PCBs ranged from 34.2% in AR to 61.2% in ONWR eggs (Fig.1). The greatest concentrations of hexa-CBs were found in MR and OR eggs, 940 ng/g and 903 ng/g, respectively. In turtle eggs from reference sites, the hexa-CBs concentrations were 215 ng/g in ONWR and 69 ng/g in LR (Table 2).
In OR eggs the most abundant were tetra-CBs, 35.1%, followed by hexa- and penta-CBs, 24.5% and 16.0%, respectively (Fig.1).

Individual PCB congeners

Concentrations of all individual PCB congeners determined in turtle eggs are presented in Appendix I. Table 3 presents concentrations of dominant PCB congeners, i.e., those with relative abundance greater than 1%. Relative proportions of dominant PCB congeners (percent of total) in turtle eggs have been shown in Fig.2. In the contaminated sites-collected eggs, the most abundant congeners were IUPAC 66, 99, 118, coeluting 132+153+105, and 163+ 138, as well as 180, 201 and coeluting 203+196. The reference sites-collected eggs showed similar accumulation patterns, however, ONWR eggs showed a dominat peak of PCBs 135+144. (Fig. 2.).

Statistical analysis of the percentage of all 89 individual peaks to total PCB in eggs with those in Aroclor 1260, Aroclor 1254 and Aroclor 1254 indicated that the distribution of PCB congeners at all collection sites was similar to that found in Aroclor 1260 and Aroclor 1254. OR eggs were highly correlated with Aroclor 1248, as well as with Aroclor 1254 (Fig. 2, Table 3). Moreover, many of the congeners showed consistently higher proportions in turtle eggs than in respective Aroclors. In most of the eggs, these isomers comprised a range of chlorination from tri- to octa-, with a dominating hexa-and hepta-CBs.

Non-ortho-CBs and TEQ concentrations

Among the non-ortho CBs, the congener number 77 occurred at the greatest concentration (Fig.3). Mean concentration ranged from 24.8 ng/g in OR eggs to 2.5 ng/g in AR eggs. The reference sites eggs had mean concentrations of 1.3 and 0.5 ng/g. The accumulation was significantly greater in OR and MR eggs than in AR and reference sites eggs. Congener number 81 was found at a similar range of concentrations as congener 126, i.e., 0.11-5.42 ng/g and 0.18-5.61 ng/g, respectively. OR had the greatest concentration of PCB 81, while PCB 126 occurred at the greatest concentration in MR eggs. There were no significant differences in PCB 169 among locations, its concentrations were the least.

TEQs are presented in Fig.4. In the TEQ calculations PCBs numbers 105,114, 123, 157 and 170 were not included. These congeners coeluted with others and, therefore, could lead to an overestimation of TEQs. The maximum TEQ concentration of 1.15 ng/g wet weight was determined in MR eggs, and the minimum of 0.041 ng/g in reference ONWR eggs. Surprisingly, the second reference LR eggs showed TEQ levels similar to that of AR and BR eggs, i.e., 0.12 ng/g and 0.16ng/g, respectively. This results from relatively greater concentrations of PCB 126 in LR eggs than in AR and BR eggs.

The ratio of TEQs to total PCBs in turtle eggs collected from contaminated sites ranged from 12.71×10^{-6} in OR to 45.94×10^{-6} in MR eggs (Table 5). These values are greater than those calculated for Aroclor 1248, Aroclor 1254 and Aroclor 1260, indicating that relative enrichment of these congeners might have taken place in turtle eggs.

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TABLE 1. Female turtle weight and eggs characteristics.

TURTLE Code	Turtle Weight (kg)	Mean Egg Weight(g) n=7-8	Dry Matter Content(%)	Lipid Content (% wet wt)
ONWR 10	8.52	15.43 ±1.10	21.82 ±0.01	7.00 ±0.12
ONWR 12	5.75	12.71 ±0.33	24.60 ±0.29	7.61 ±0.08
ONWR 13	9.40	11.86 ±1.00	25.59 ±0.05	7.64
ONWR 14	7.10	13.71 ±0.57	20.66 ±0.17	7.98 ±0.27
LR 1	5.70	11.39 ±0.32	28.41 ±0.08	9.31 ±0.11
LR 2	8.05	13.66 ±0.21	23.84 ±0.06	7.94 ±0.12
LR 3	4.42	11.26 ±0.26	21.82 ±0.04	7.11 ±0.40
LR 6	3.52	10.25 ±0.44	22.22 ±0.01	7.56 ±0.15
OR 6[(A)]	3.50	Immature eggs	46.31 ±0.01	14.42 ±0.17
OR 8	4.40	9.60 ±0.42	24.77 ±0.03	7.57 ±0.16
OR 11	3.68	6.76 ±0.56	23.21 ±0.01	7.36 ±0.04
MR 1	4.47	8.75 ±0.51	24.66 ±0.12	7.47 ±0.29
MR 2	4.78	9.74 ±0.23	24.72 ±0.01	8.39 ±0.07
AR 5	7.95	11.13 ±0.27	25.95 ±0.05	8.52 ±0.20
AR 10	4.50	11.89 ±0.53	25.22 ±0.16	8.40 ±0.05
BR 3	6.68	12.04 ±0.21[(B)]	26.58 ±0.01	8.70 ±0.08

(A) OR6 female had small, immature eggs that did not have a shell or egg whites developed.

(B) BR3 sample includes 3 eggs.

TABLE 2. Total PCBs and PCB homologue concentrations (ng/g wet wt) in turtle eggs (mean±SD).

Collection site	ONWR	LR	OR	MR	AR	BR
No. of turtles	3	4	3	2	2	1
No. of eggs analyzed/turtle	7-8	7-8	7-8	7-8	7-8	3
Tri-CBs	4.34±0.82	3.40±0.6	203.53±104.91	42.61±31.09	87.45±34.38	18.59
Tetra-CBs	11.26±1.72	12.65±5.36	1292.24±530.42	308.49±218.45	152.36±55.46	86.23
Penta-CBs	22.70±4.08	19.54±9.65	587.71±253.99	362.29±197.17	153.24±20.54	151.53
Hexa-CBs	215.73±174.73	69.81±46.81	903.21±404.06	940.20±352.36	369.25±46.70	350.94
Hepta-CBs	58.37±15.91	44.85±35.56	392.56±171.67	566.64±232.66	198.32±12.85	167.06
Octa-CBs	37.39±13.06	30.69±24.11	291.05±185.10	278.78±81.59	110.36±6.81	91.93
Nona-CBs	2.68±1.09	2.29±1.82	13.07±5.94	19.97±5.57	9.21±0.72	7.11
Deca-CBs	0.02±0.01	0.02±0.01	0.06±0.05	0.11±0.02	0.11±0.11	0.07
Total PCBs	352.5±160.4[ac]	183.24±121.85[a]	3683.43±1456.10[b]	2519.09±1118.90[b]	1080.30±177.57[cb]	873.45[cb]

Means for total PCBs that do not share the same letter (a, b, c, or d) are significantly different ($p<0.001$).

TABLE 3. Concentrations of dominant individual PCB congeners (ng/g wet weight, N=number of females, n=number of eggs analyzed/female).

	Collection site										
PCB	ONWR		LR		OR		MR		AR		BR
IUPAC	N=3		N=4		N=3		N=2		N=2		N=1
Number	n=7-8		n=7-8		n=7-8		n=7-8		n=7-8		n=3
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
26	0.257	0.116	0.247	0.122	14.379	7.539	4.641	1.765	53.371	22.652	1.641
31+28	1.910	0.506	1.048	0.616	128.219	51.606	21.289	18.624	4.485	1.799	2.990
47+48	2.246	0.780	2.054		39.033	18.255	12.742	5.973	5.943		7.210
74	0.718	0.074	1.042	0.485	215.529	81.061	44.920	31.556	31.805	6.947	9.858
66	1.879	0.176	4.680	3.047	741.126	293.234	170.755	128.626	73.875	26.079	28.671
56+60	0.843	0.085	0.869	0.313	193.636	82.522	35.533	25.089	30.867	10.069	7.074
95	1.093	0.580	0.750	0.122	12.893	7.668	9.473	4.035	2.648	1.773	10.889
92+84	1.507	0.780	1.573	0.503	56.872	29.932	43.738	27.258	8.941	4.587	18.071
99	2.562	0.728	3.387	1.527	93.737	34.272	65.135	35.630	26.871	6.025	24.429
85	0.558	0.092	0.897	0.455	60.360	27.278	36.225	25.333	10.931	3.895	8.280
118	13.517	3.955	9.905	6.400	276.117	104.130	142.259	64.797	87.407	0.901	58.140
151	0.396	0.128	0.935	0.539	14.896	12.223	27.638	23.629	4.341	1.343	4.017
135+144	109.895	189.535	0.708	0.275	11.923	5.797	20.143	13.307	4.723	1.116	5.069
114+131	1.936	0.532	0.991	0.764	112.576	57.691	25.678	15.834	16.841	3.743	12.246
146	2.218	0.686	2.252	1.014	35.939	15.843	57.080	33.498	16.613	1.112	14.444
132+153+	41.022	9.881	24.941	17.189	304.049	114.161	312.242	119.296	136.203	2.712	105.651
163+138	53.830	14.991	33.855	23.722	314.377	150.426	399.698	114.865	151.328	46.270	161.911
158	2.531	0.830	2.474	2.138	39.416	18.010	44.515	24.437	13.396	1.461	14.928
167	1.224	1.482	0.204	0.156	22.495	8.147	1.551	0.551	6.891	8.313	9.910
178	3.627	1.350	3.147	2.475	26.236	11.157	41.622	17.550	14.491	1.548	12.143
187+182	2.875	0.908	3.459	2.113	40.135	19.078	62.079	32.525	18.719	2.699	15.484
183	6.749	1.644	4.038	3.400	37.950	15.338	55.985	20.933	20.207	0.317	17.322
174	0.551	0.154	1.035	0.541	13.982	8.329	26.891	18.771	4.375	1.181	5.481
177	1.019	0.384	2.301	1.750	25.439	13.621	37.935	23.517	8.841	1.637	9.262
172	2.923	0.974	2.551	2.120	22.881	11.076	33.364	13.889	12.395	0.984	10.494
180	28.674	7.534	19.157	16.260	137.119	52.504	190.406	59.635	75.899	2.241	62.112
170+190	8.336	2.316	5.548	4.675	51.147	20.443	67.438	28.814	25.865	1.589	21.247
201	9.765	3.932	9.970	7.198	158.243	178.697	78.139	22.577	30.872	3.954	25.346
203+196	22.266	7.604	16.888	13.923	101.400	42.098	156.918	43.078	63.013	2.653	52.325
194	3.128	0.915	2.209	1.977	16.320	6.628	22.062	7.683	8.910	0.471	7.305

TABLE 4. Comparison of PCB congener profiles in turtle eggs with that in Aroclors, Pearson correlation probabilities (Systat).

Sampling site	Aroclor 1260	Aroclor 1254	Aroclor 1248
ONWR	P=0.028	P=0.062	P=0.481
LR	P=0.000	P=0.000	P=0.781
OR	P=0.185	P=0.000	P=0.001
MR	P=0.000	P=0.000	P=0.708
AR	P=0.001	P=0.000	P=0.516
BR	P=0.000	P=0.000	P=0.850

TABLE 5. Ratio of TEQs to total PCBs in turtle eggs.

Collection site	Ratio of TEQs to total PCBs $\times 10^{-6}$
ONWR	11.83
LR	70.80
OR	12.71
MR	45.94
AR	14.81
BR	18.67
Aroclor 1248[A]	12.4
Aroclor 1254[A]	10.8
Aroclor 1260[A]	3.2

[A] Data from Giesy et al 1997.

Fig. 1. PCB homologues content.

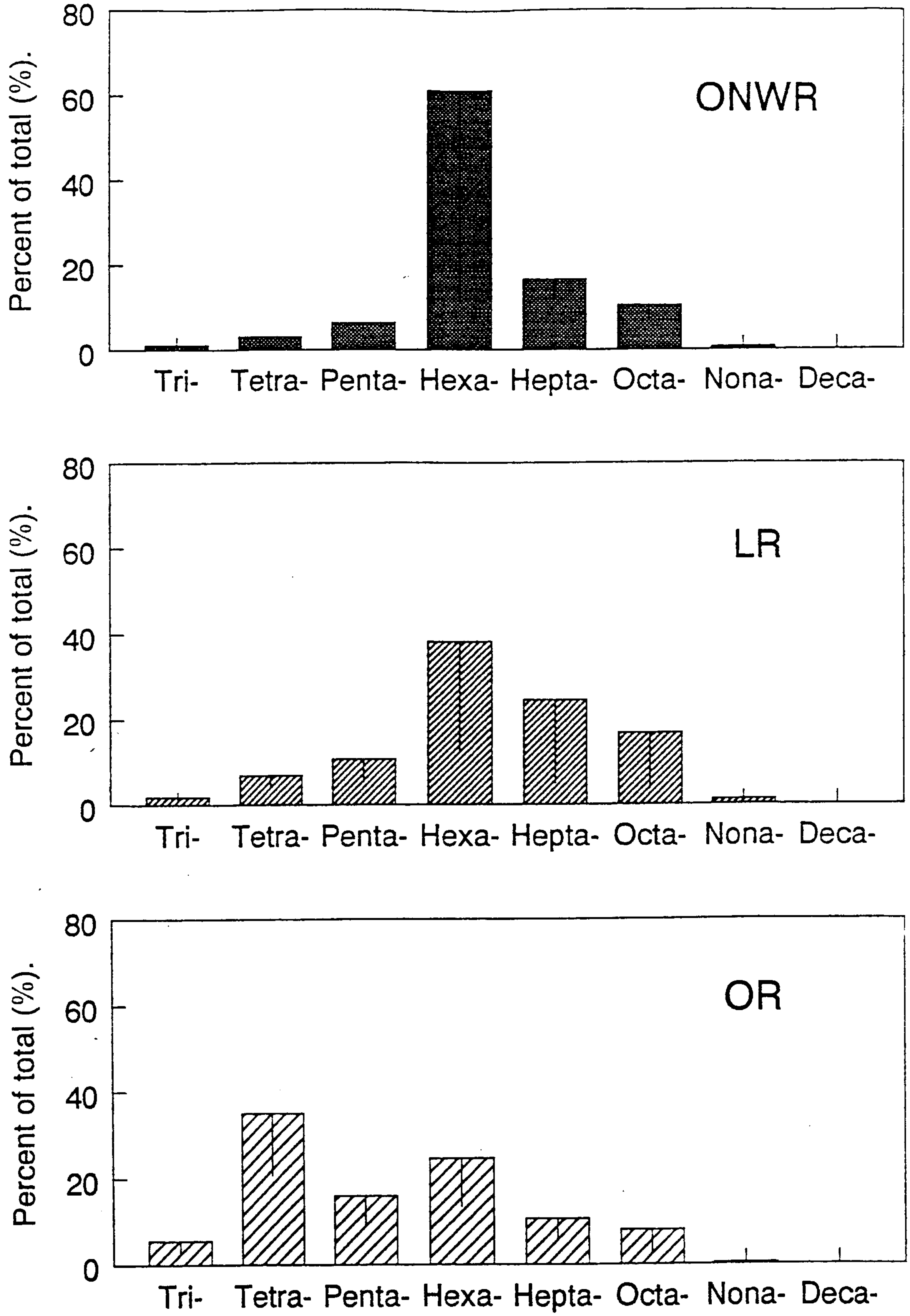
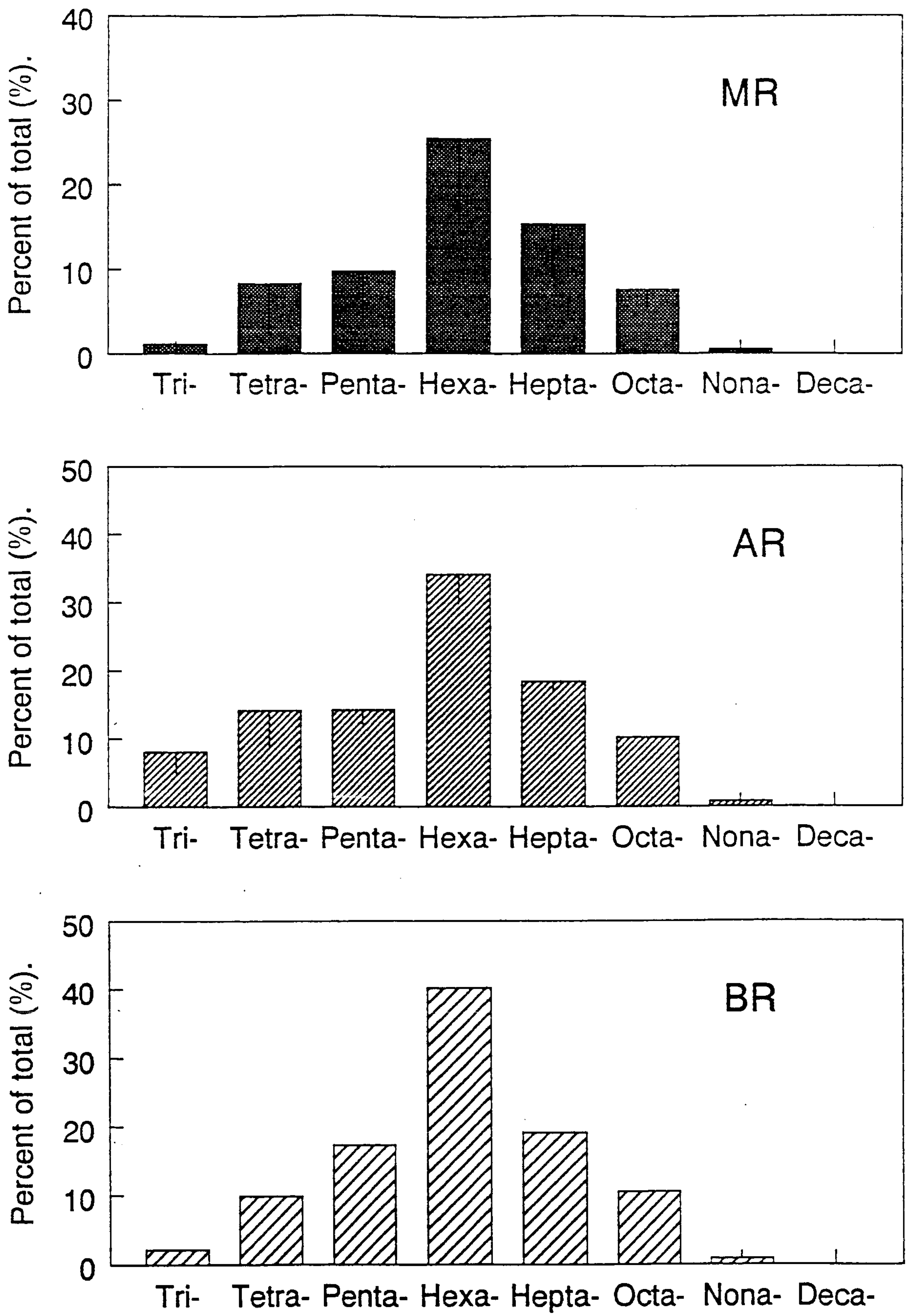
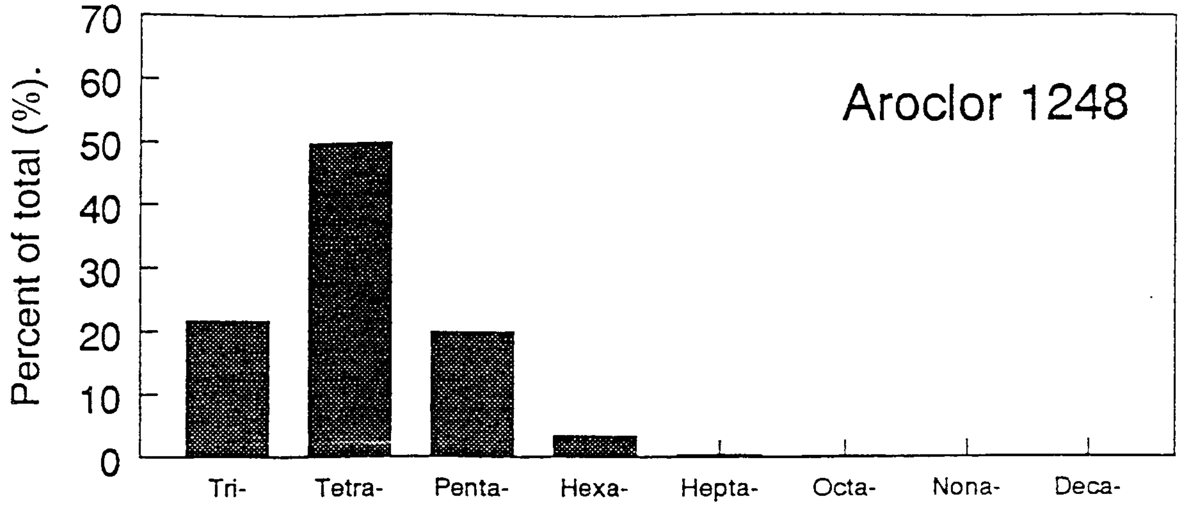


Fig.1. Cont.

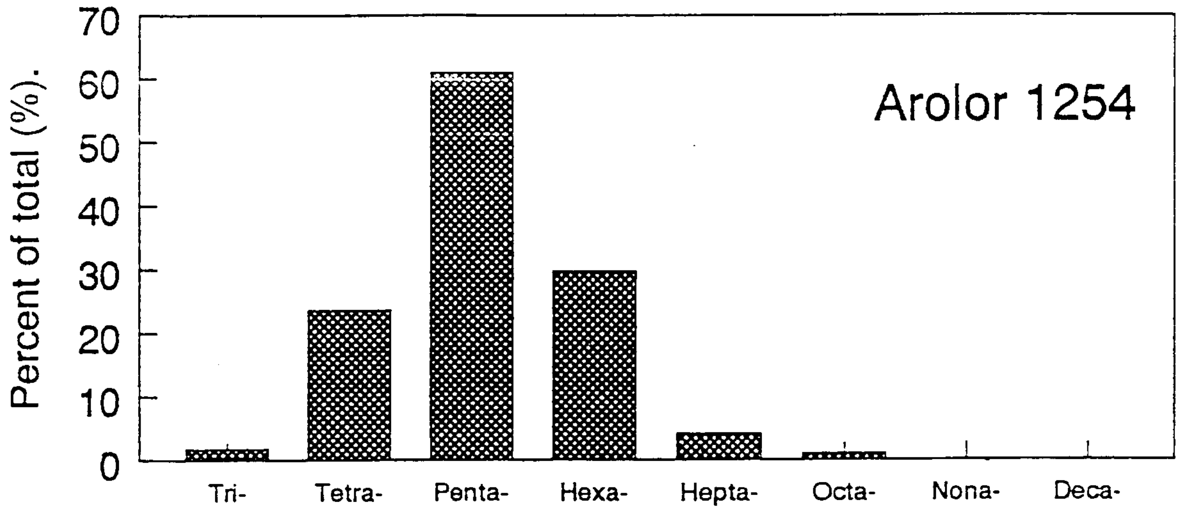


MR
AR
BR
Percent of total (%).
0
10
20
30
40
50
Tri-
Tetra-
Penta-
Hexa-
Hepta-
Octa-
Nona-
Deca-

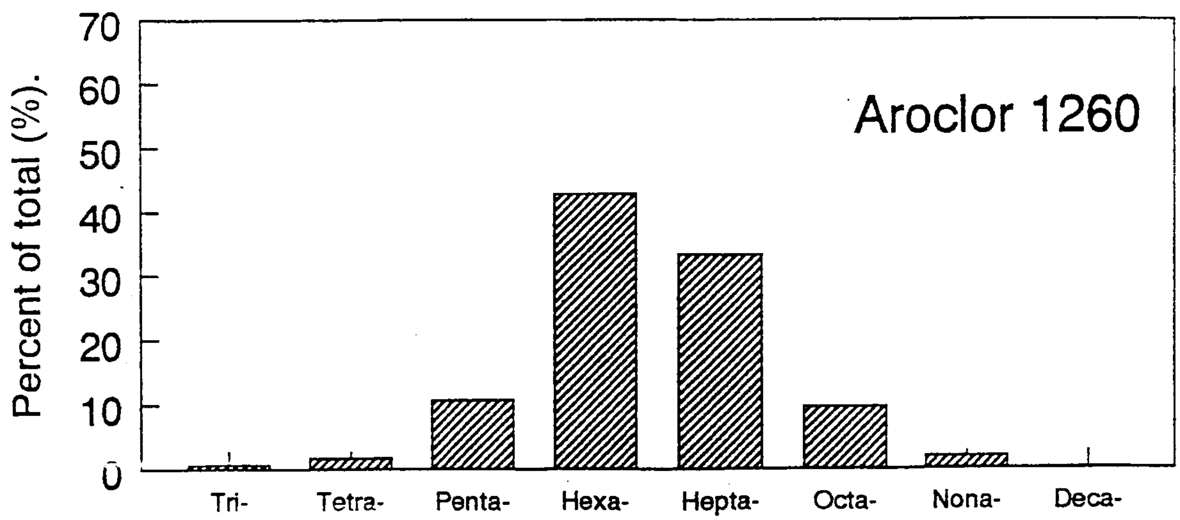
Fig. 1. Cont.



Aroclor 1248
Percent of total (%).
70
60
50
40
30
20
10
0
Tri-
Tetra-
Penta-
Hexa-
Hepta-
Octa-
Nona-
Deca-



Arolor 1254
Percent of total (%).
70
60
50
40
30
20
10
0
Tri-
Tetra-
Penta-
Hexa-
Hepta-
Octa-
Nona-
Deca-



Aroclor 1260
Percent of total (%).
70
60
50
40
30
20
10
0
Tri-
Tetra-
Penta-
Hexa-
Hepta-
Octa-
Nona-
Deca-

Fig. 2. Relative composition of PCB isomers in turtle eggs.

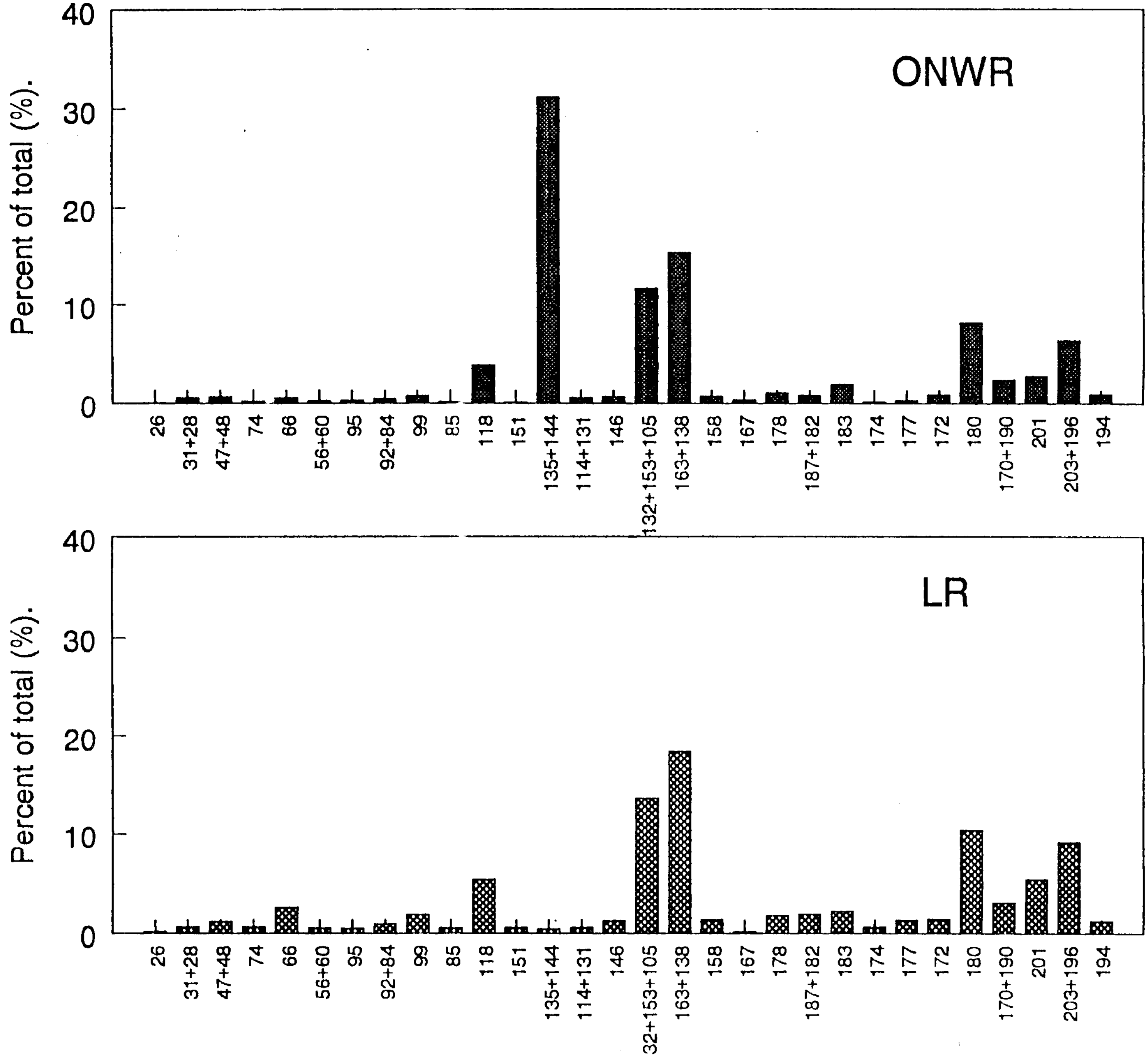
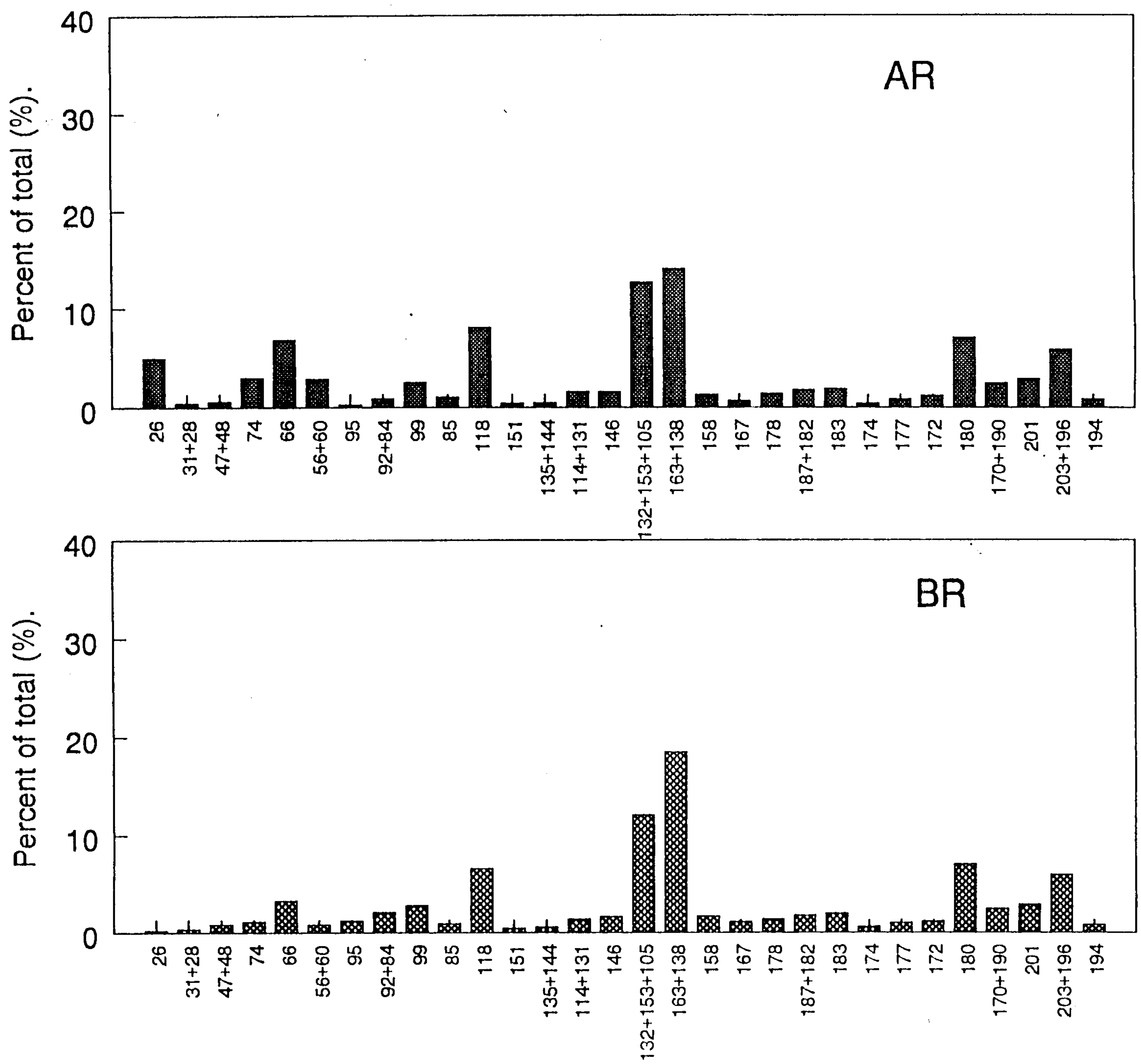


Fig. 2. Cont..



AR
BR
Percent of total (%).
0
10
20
30
40
26
31+28
47+48
74
66
56+60
95
92+84
99
85
118
151
135+144
114+131
146
132+153+105
163+138
158
167
178
187+182
183
174
177
172
180
170+190
201
203+196
194

Fig. 2. Cont..

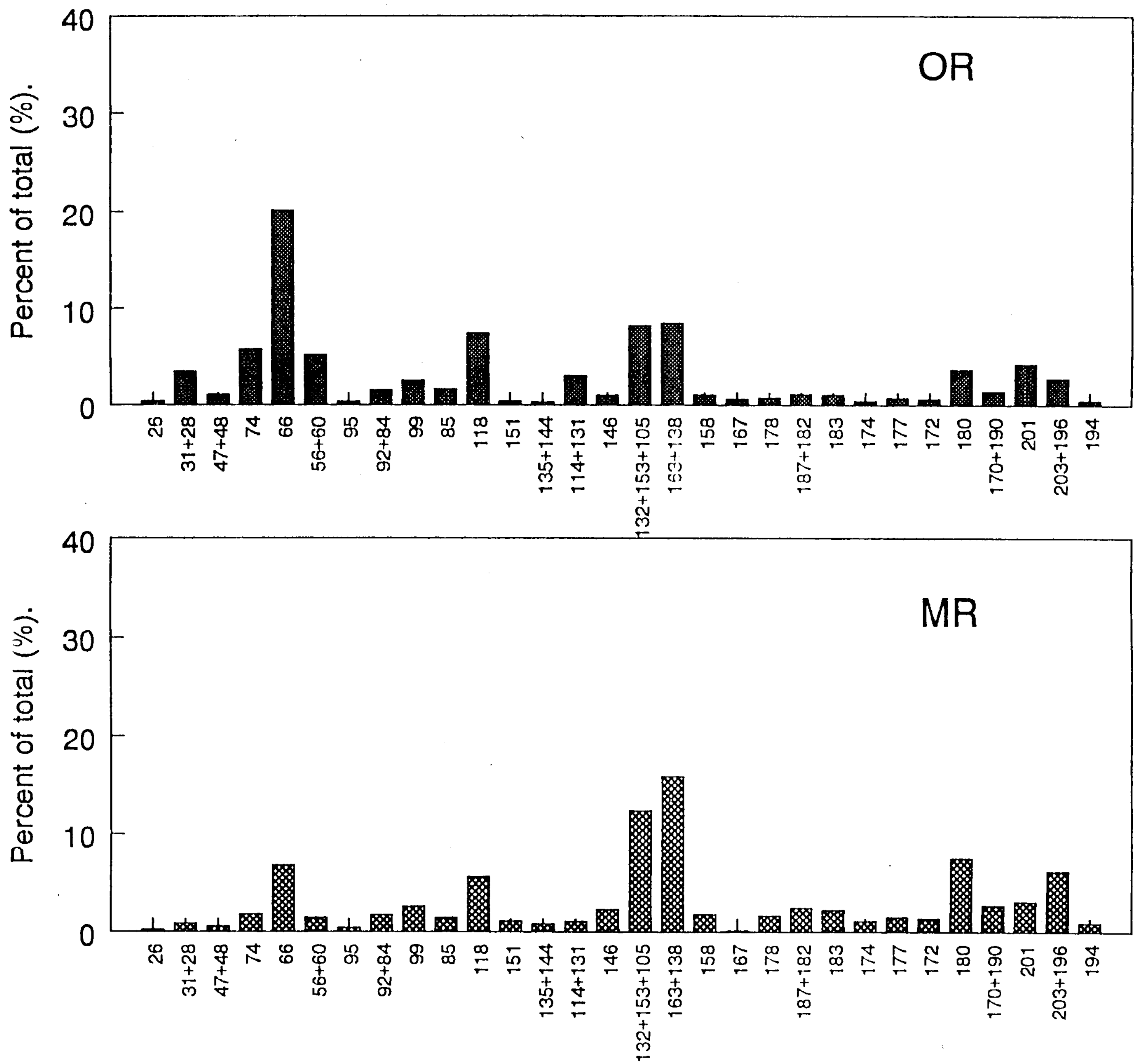
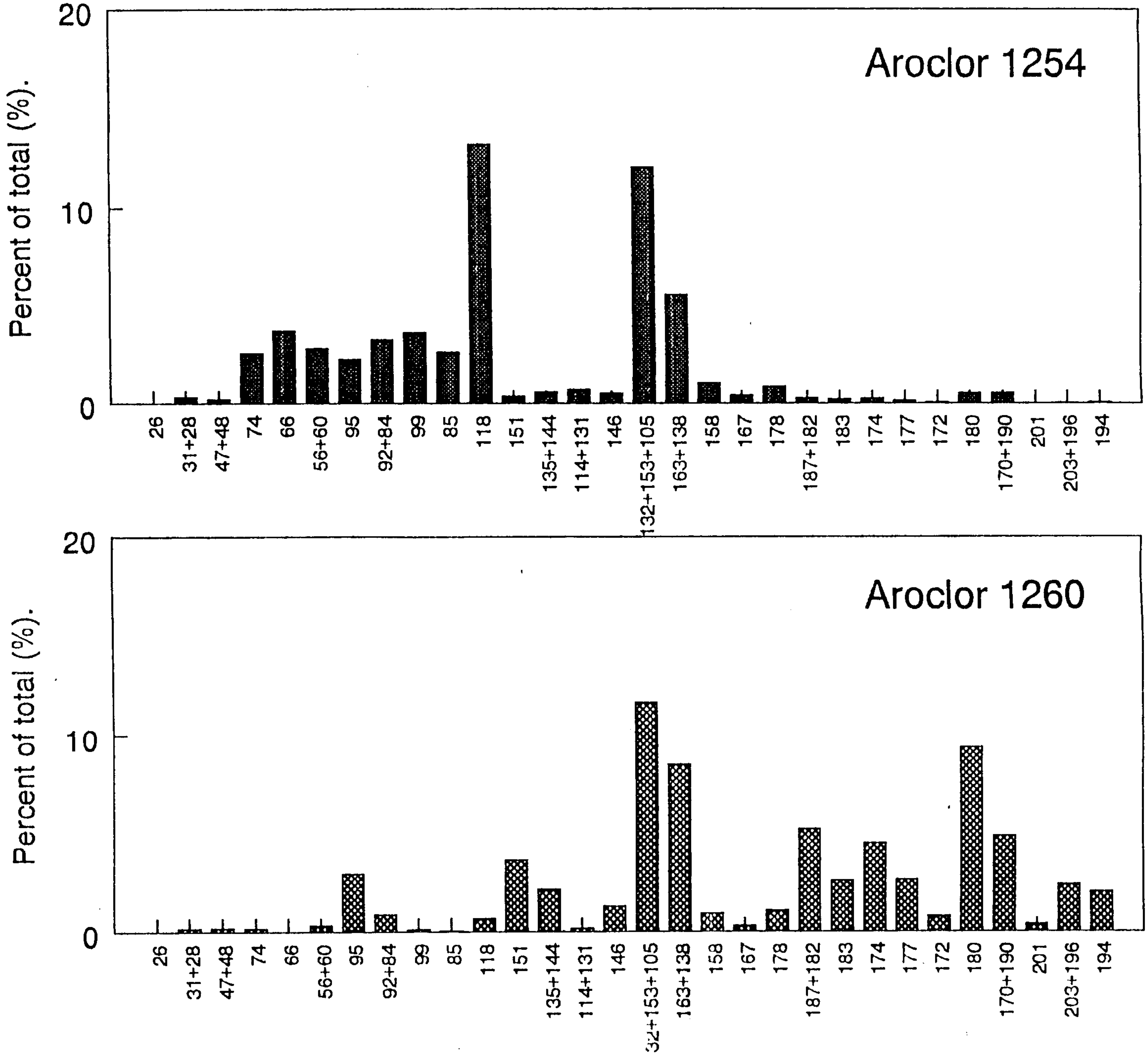

OR
Percent of total (%).
40
30
20
10
0
26
31+28
47+48
74
66
56+60
95
92+84
99
85
118
151
135+144
114+131
146
132+153+105
163+138
158
167
178
187+182
183
174
177
172
180
170+190
201
203+196
194
MR
Percent of total (%).
40
30
20
10
0
26
31+28
47+48
74
66
56+60
95
92+84
99
85
118
151
135+144
114+131
146
132+153+105
163+138
158
167
178
187+182
183
174
177
172
180
170+190
201
203+196
194

Fig. 2. Cont.


Aroclor 1254
Percent of total (%).
20
10
0
26
31+28
47+48
74
66
56+60
95
92+84
99
85
118
151
135+144
114+131
146
132+153+105
163+138
158
167
178
187+182
183
174
177
172
180
170+190
201
203+196
194
Aroclor 1260
Percent of total (%).
20
10
0

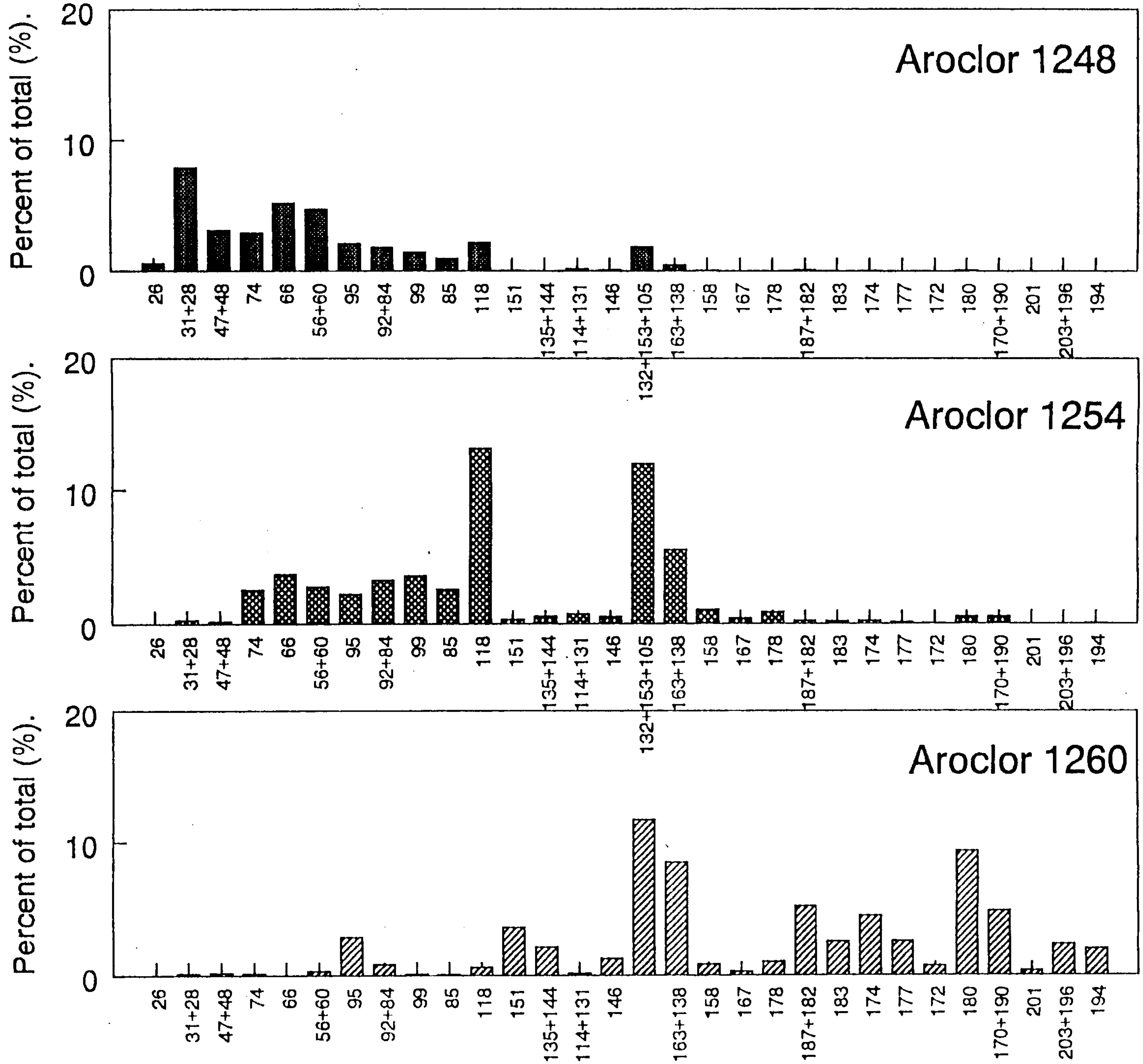
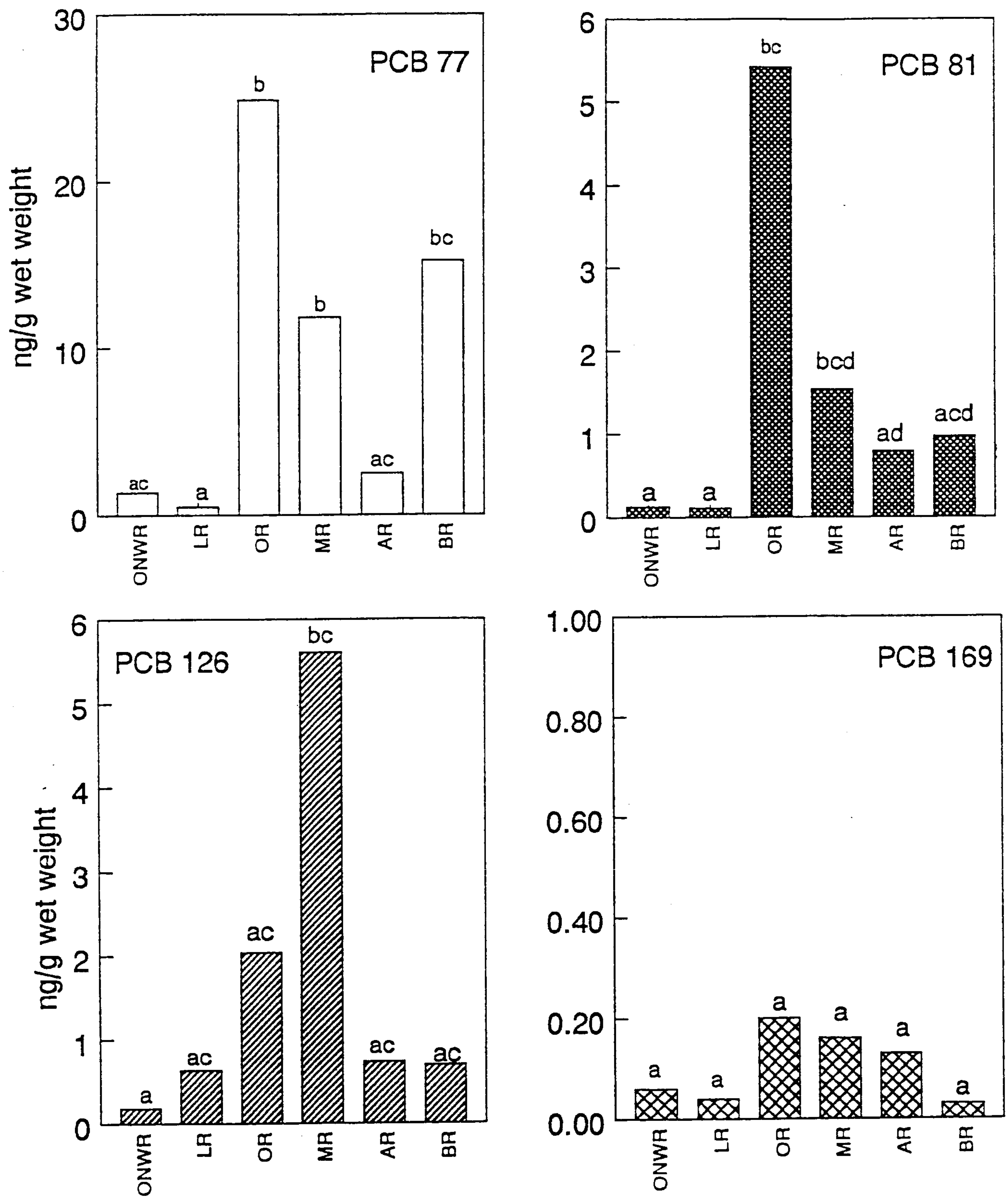
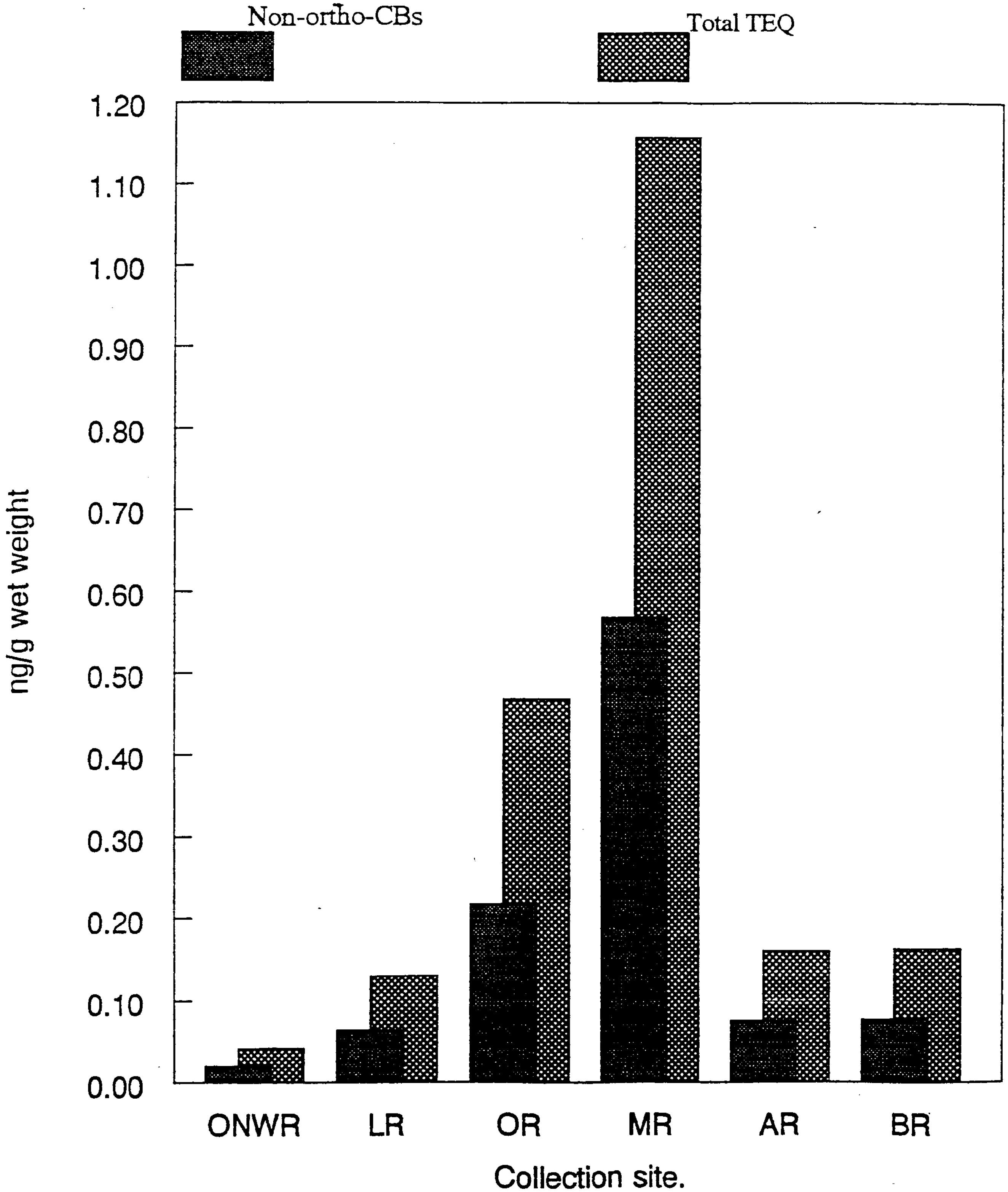

Aroclor 1248
Aroclor 1254
Aroclor 1260
Percent of total (%).
0
10
20
26
31+28
47+48
74
66
56+60
95
92+84
99
85
118
151
135+144
114+131
146
132+153+105
163+138
158
167
178
187+182
183
174
177
172
180
170+190
201
203+196
194

Fig. 3. Non-ortho-CBs in turtle eggs (mean, ng/g wet wt).



Values that do not share the same letter are sigificantly different (p<0.05).

Fig. 4. Concentrations of TEQs.



TEQs were determined from concentrations of non-ortho-CBs and some mono- and di- CBs.

Appendix I. Concentrations of individual PCB congeners determined in turtle eggs (ng/g wet weight).

P C B											
IUPAC	ONWR		LR		OR		MR		AR		BR 3
NUMBER	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	
19	0.047	0.030	0.023	0.007	0.363	0.279	0.115	0.022	0.060	0.008	0.360
18	0.473	0.067	0.323	0.059	4.460	2.628	1.935	1.794	3.504		1.405
15+17	0.551	0.614	0.634	0.052	24.204	20.296	5.314	2.208	23.368	11.396	5.834
24+27	0.031	0.019	0.026	0.002	0.525	0.358	0.213	0.227	0.064	0.043	0.266
16	0.251	0.215	0.211	0.060	5.959	3.529			0.370		2.662
32	0.191	0.021	0.386	0.136	1.792		1.366	0.507	0.553	0.001	
26	0.257	0.116	0.247	0.122	14.379	7.539	4.641	1.765	53.371	22.652	1.641
25	0.086		0.076		3.488	2.233	3.488		0.691	0.300	0.223
31+28	1.910	0.506	1.048	0.616	128.219	51.606	21.289	18.624	4.485	1.799	2.990
33	0.501	0.153	0.523	0.156	17.318	14.965	4.447	2.364	2.158	0.714	2.329
22	0.480		0.323	0.022	6.001	3.838	1.542	1.113	0.761	0.289	0.879
SUM-tri	4.337		3.400		203.526		42.605		87.448		18.589
53	0.056	0.054	0.068	0.018	0.683	0.463	0.187	0.184	0.075	0.068	0.273
51	0.062	0.023	0.019	0.006	0.223	0.160	0.072	0.002	0.028	0.029	0.083
45	0.108	0.060	0.083	0.020	1.032	0.775	0.309	0.220	0.061	0.003	0.549
46	0.077	0.041	0.072	0.062	0.357	0.290	0.207	0.070	0.953		0.276
52	1.430	0.661	0.886	0.335	22.500	13.120	10.535	8.411	2.113	1.813	11.525
43	0.134	0.047	0.170	0.200	1.488	1.265	1.751	1.714	0.251	0.153	0.417
49	0.376	0.040	0.312	0.173	11.448	6.371	4.820	3.928	0.795	0.615	1.882
47+48	2.246	0.780	2.054		39.033	18.255	12.742	5.973	5.943		7.210
44	0.894	0.215	1.206	0.474	11.177	5.934	5.269	3.162	1.413	0.932	5.343
37+42	1.010	0.121	1.178	0.588	7.640	4.753	3.792	1.767	1.768	0.056	2.493
41+71	1.008	0.184	0.718	0.303	20.857	13.697	7.220	3.231	2.673	1.137	3.969
64	0.289	0.099	0.203	0.131	3.637		2.412	1.051	0.588	0.831	0.920
40	0.170	0.067	0.080	0.029	1.037	0.759	0.447	0.189	0.177	0.085	0.522
63	0.105	0.078	0.109	0.049	4.398	3.897	0.465	0.022	0.170	0.167	0.233
74	0.718	0.074	1.042	0.485	215.529	81.061	44.920	31.556	31.805	6.947	9.858
70+76	0.477	0.180	0.329	0.068	13.448	6.901	5.516	4.300	1.466	0.990	3.968
66	1.879	0.176	4.680	3.047	741.126	293.234	170.755	128.626	73.875	26.079	28.671
56+60	0.843	0.085	0.869	0.313	193.636	82.522	35.533	25.089	30.867	10.069	7.074
81	0.127	0.040	0.112	0.021	5.416	3.919	1.535	1.007	0.785	0.617	0.965
SUM-tet	11.261		12.648		1292.24		308.487		152.358		86.230
100	0.117	0.059	0.057	0.041	1.325	0.856	0.855	0.305	0.402	0.138	0.145
95	1.093	0.580	0.750	0.122	12.893	7.668	9.473	4.035	2.648	1.773	10.889
91	0.207	0.107	0.095	0.022	3.529	2.187	2.494	1.634	0.437	0.232	1.934
92+84	1.507	0.780	1.573	0.503	56.872	29.932	43.738	27.258	8.941	4.587	18.071
89	0.167	0.081	0.309	0.256	1.154	0.719	1.604	0.013	1.178	0.433	1.214
101	1.061	0.323	0.792	0.185	12.251	7.663	15.949	10.238	2.678	1.437	8.242
99	2.562	0.728	3.387	1.527	93.737	34.272	65.135	35.630	26.871	6.025	24.429
119	0.039	0.024	0.071	0.053	4.232	3.318	1.504	0.926	0.262	0.220	0.256
83	0.132	0.024	0.041	0.019	1.933	1.456	1.459	1.004	0.250	0.168	0.612
97	0.306	0.072	0.185	0.121	2.120	1.789	2.444	1.054	0.726	0.348	1.396
87	0.398	0.170	0.262	0.084	9.106	3.932	5.512	2.874	1.680	0.518	4.033
85	0.558	0.092	0.897	0.455	60.360	27.278	36.225	25.333	10.931	3.895	8.280

110	0.658	0.428	0.570	0.153	14.163	8.333	15.055	10.322	2.114	1.113	8.023
82	0.136	0.046	0.093	0.063	1.450	1.292	1.366	0.435	0.461	0.168	1.080
107	0.242	0.204	0.549	0.293	36.467	21.780	17.219	11.313	6.252	0.380	4.783
118	13.517	3.955	9.905	6.400	276.117	104.130	142.259	64.797	87.407	0.901	58.140
SUM-pen	22.702		19.537		587.709		362.290		153.239		151.529
136	0.210	0.041	0.208		2.436	1.461	2.482	1.285	0.961		1.367
151	0.396	0.128	0.935	0.539	14.896	12.223	27.638	23.629	4.341	1.343	4.017
135+144	109.895	189.535	0.708	0.275	11.923	5.797	20.143	13.307	4.723	1.116	5.069
123+149	0.723	0.138	0.982	0.353	11.228	4.804	24.820	14.169	4.039	1.409	7.197
134	0.052	0.023	0.045	0.018	0.683	0.499	1.180	0.789	0.216	0.116	0.417
114+131	1.936	0.532	0.991	0.764	112.576	57.691	25.678	15.834	16.841	3.743	12.246
146	2.218	0.686	2.252	1.014	35.939	15.843	57.080	33.498	16.613	1.112	14.444
132+153	41.022	9.881	24.941	17.189	304.049	114.161	312.242	119.296	136.203	2.712	105.651
141	0.185	0.035	0.360	0.173	4.950	3.563	11.153	8.754	1.607	0.473	1.955
130	0.618	0.320	1.162	0.699	21.207	10.649	7.862	11.118	10.045	0.629	7.745
163+138	53.830	14.991	33.855	23.722	314.377	150.426	399.698	114.865	151.328	46.270	161.911
158	2.531	0.830	2.474	2.138	39.416	18.010	44.515	24.437	13.396	1.461	14.928
129	0.004	0.004	0.006	0.004	0.049	0.027	0.033	0.047	0.025	0.009	0.028
128	0.013	0.005	0.005	0.004	0.163	0.113	0.053	0.021	0.038	0.002	0.092
167	1.224	1.482	0.204	0.156	22.495	8.147	1.551	0.551	6.891	8.313	9.910
156	1.312	0.452	0.843	0.628	7.631	6.912	8.154		2.465	2.678	3.960
SUM-hex	215.732		69.814		903.205		940.204		369.251		350.937
137+176	0.476	0.150	0.278	0.220	4.067	1.857	4.999	2.252	1.904	0.225	
178	3.627	1.350	3.147	2.475	26.236	11.157	41.622	17.550	14.491	1.548	12.143
175	0.219	0.074	0.246	0.166	4.043	2.669	6.482	3.983	1.548	0.415	1.464
187+182	2.875	0.908	3.459	2.113	40.135	19.078	62.079	32.525	18.719	2.699	15.484
183	6.749	1.644	4.038	3.400	37.950	15.338	55.985	20.933	20.207	0.317	17.322
185	0.048	0.020	0.082	0.043	1.364	1.069	3.108	2.426	0.454	0.123	0.502
174	0.551	0.154	1.035	0.541	13.982	8.329	26.891	18.771	4.375	1.181	5.481
177	1.019	0.384	2.301	1.750	25.439	13.621	37.935	23.517	8.841	1.637	9.262
173			0.076	0.027	0.876	0.713	0.852	0.204	0.379	0.086	0.235
172	2.923	0.974	2.551	2.120	22.881	11.076	33.364	13.889	12.395	0.984	10.494
180	28.674	7.534	19.157	16.260	137.119	52.504	190.406	59.635	75.899	2.241	62.112
193	1.507	0.471	1.355	1.112	11.738	6.444	16.711	4.449	5.804	1.387	5.453
191	1.239	0.425	1.543	1.017	13.374	7.962	17.156	1.997	6.842	2.180	4.903
170+190	8.336	2.316	5.548	4.675	51.147	20.443	67.438	28.814	25.865	1.589	21.247
189	0.132	0.127	0.050	0.041	2.211	0.889	1.611	1.717	0.602	0.580	0.958
SUM-hep	58.374		44.846		392.562		566.637		198.323		167.059
202+171	1.553	0.468	1.111	0.837	9.874	4.260	14.833	5.382	5.175	0.583	4.609
157+200	0.386	0.136	0.227	0.146	1.923	1.226	2.442	0.334	0.964	0.339	0.869
199	0.046	0.019	0.089	0.009	0.899	0.581	1.601	0.991	0.354	0.072	0.391
198	0.086	0.040	0.087	0.066	0.782	0.402	1.200	0.524	0.428	0.020	0.334
201	9.765	3.932	9.970	7.198	158.243	178.697	78.139	22.577	30.872	3.954	25.346
03+196	22.266	7.604	16.888	13.923	101.400	42.098	156.918	43.078	63.013	2.653	52.325
194	3.128	0.915	2.209	1.977	16.320	6.628	22.062	7.683	8.910	0.471	7.305
205	0.160	0.059	0.111	0.086	1.611	0.650	1.589	1.021	0.649	0.340	0.749

SUM-oct	37.390		30.693		291.053		278.785		110.364		91.927
208+195	1.286	0.493	0.985	0.839	6.413	2.875	9.467	2.618	3.853	0.327	3.036
207	0.402	0.185	0.328	0.261	2.273	1.201	3.771	1.245	1.667	0.148	1.293
206	0.991	0.420	0.975	0.722	4.386	1.864	6.731	1.709	3.684	0.248	2.779
SUM-non	2.679		2.289		13.072		19.970		9.205		7.108
209	0.022	0.012	0.017	0.012	0.061	0.049	0.112	0.018	0.113	0.109	0.070
Total											
ng/g	352.497		183.24		3683.43		2519.09		1080.30		873.45