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Concentrations and patterns of organochlorine contaminants in marine turtles from Mediterranean and Atlantic waters

C. Mckenzie [a,b,*], B.J. Godley [c,d], R.W. Furness [c], D.E. Wells [a]

[a] *FRS Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB11 9DB, UK*
[b] *School of Applied Sciences, Robert Gordon University, St Andrews Street, Aberdeen AB10 1FR, UK*
[c] *Institute of Biomedical and Life Sciences, Graham Kerr Building, University of Glasgow, Glasgow G12 8QQ, UK*
[d] *Department of Veterinary Anatomy, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, UK*

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Abstract

Concentrations of individual chlorobiphenyls (CBs) and organochlorine pesticides (OCPs) in marine turtle tissues collected from the Mediterranean (Cyprus, Greece) and European Atlantic waters (Scotland) between 1994 and 1996 are described. ΣCB concentrations were highest in adipose tissue and ranged from 775 to 893, 39 to 261 and 47 to 178 μg/kg wet wt in loggerhead (*Caretta carretta*), green (*Chelonia mydas*) and leatherback (*Dermochelys coriacea*) turtles, respectively. Omnivorous loggerhead turtles had the highest organochlorine contaminant (OC) concentrations in all tissues sampled. It is thought that dietary preferences were likely to be the main differentiating factor among species. Decreasing lipid contaminant burdens with turtle size were observed in green turtles, most likely attributable to a change in diet with age. Principal component analysis of data from loggerhead and green turtles indicated that there were also pattern differences between species, confirming bioaccumulation differences.

Keywords: Marine turtles; *Caretta carretta*; *Chelonia mydas*; *Dermochelys coriacea*; Organochlorine pesticides

* Corresponding author.

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1. Introduction

The three sea turtle species most commonly found in Mediterranean and European Atlantic waters are the green turtle (*Chelonia mydas*), the loggerhead turtle (*Caretta caretta*) and the leatherback turtle (*Dermochelys coriacea*) (Brongersma, 1972; Groombridge, 1990). Only the green turtle and the loggerhead turtle are thought to breed in the Mediterranean region, with nesting mainly taking place on the shores of the eastern Mediterranean (Broderick and Godley, 1996), both populations being considered as regionally endangered. In addition, marine turtles from distant breeding populations, especially leatherback and loggerhead turtles, are often recorded in the European Atlantic and the Mediterranean. These species are considered as globally endangered and globally threatened, respectively.

An extensive review of environmental contaminants in turtles has been conducted by Meyers-Schöne and Walton (1994); however, most information relates to freshwater species. Studies have found a mean concentration of organochlorine contaminants (OCs) in the freshwater species, snapping turtle (*Chelydra serpentina*) from the Hudson river, of 2990 μg/g in its adipose tissue. High concentrations of chlorobiphenyls (CBs) and organochlorine pesticides (OCPs) in the eggs of this species have been correlated with population effects such as decreased hatching success, increased hatchling deformities and disorientation (Bishop et al., 1991, 1994). There have been few studies on the concentrations and bioaccumulation of OCs in marine turtles. In the majority of cases reported only the total CB concentrations have been provided, with no information about the congener profile. Clark and Krynitsky (1980) measured CBs in loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) eggs. CB concentrations in the eggs of loggerhead turtles ranged from 32 to 201 μg/kg and were below detectable limits in those of the green turtles. Loggerhead turtles from the east coast of Florida contained < 5.0–133 μg/kg Arochlor equivalents in their livers whilst green turtles from the same area had liver CB burdens ranging from < 5.0 to 70 μg/kg Arochlor equivalents.

Levels of contaminants have been the subject of prior concern in other megafaunal taxa in the Mediterranean. Cetaceans, particularly striped (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiops truncatus*), from the western Mediterranean have been shown to contain high organochlorine levels ranging from 90 to 1000 μg/g for ΣCB (Aguilar and Borrell, 1995; Corsolini et al., 1995). These levels have been tentatively linked with the 1990/1991 striped dolphin epizootic in the Mediterranean (Aguilar and Borrell, 1995). The only study of top predators in Greek waters found lower concentrations in a striped dolphin and a monk seal (*Monachus monachus*) from the South Aegean sea (Georgakopoulou-Gregoriadou et al., 1995).

The diet preferences of each of the sea turtle species sampled are markedly different and have been recently reviewed (Bjorndal, 1997). Loggerhead turtles worldwide are generally carnivorous, their diet thought to be dominated by benthic invertebrates. This has recently been supported by a dietary study carried out on two individuals from the eastern Mediterranean which were found to have been feeding on benthic molluscs and crustaceans (Godley et al., 1997). Leatherback turtles are

the most pelagic of the three species studied and their diet consists almost exclusively of jellyfish, salps and other gelatinous organisms. Juvenile green turtles are omnivorous but as they mature their diet becomes herbivorous, consisting mainly of sea grass.

There are similarities in the life history of the species involved (Musick and Limpus, 1997). After hatching on the natal beach, marine turtles undergo a poorly understood pelagic life history phase, where they are thought to feed upon planktonic items for a number of years. Following this, they migrate into a juvenile developmental habitat, usually demersal and neritic, where it is likely that they remain until they reach adulthood. It is during this post-pelagic phase that species appear to develop a greater degree of dietary specialisation.

In this study, 15 marine turtles comprising three species were analysed for 22 CB congeners and 17 OCPs. In addition, OC concentrations were determined in a small number of Mediterranean loggerhead and green turtle hatchlings and an egg from each species.

2. Materials and methods

2.1. Sampling

Samples of liver and adipose tissue from stranded turtles from Cyprus, Greece and Scotland were collected for organochlorine analysis. In addition, a small number of dead hatchlings and undeveloped eggs were collected from nests hatched at Alagadi, northern Cyprus, according to an established protocol (Broderick and Godley, 1996). Individual samples were wrapped in aluminium foil and transported frozen.

2.2. Analytical methodology

Tissue samples, whole hatchlings or eggs were macerated, mixed with anhydrous sodium sulphate and extracted using Soxhlet apparatus for 8 h with methyl tertiary butyl ether (300 ml). Following extraction the percentage extractable lipid was determined gravimetrically.

An aliquot containing ca 200 mg of lipid was removed from the bulk extract and transferred to hexane. The lipids were removed using a 6-g alumina column (5% H_2O) and the organochlorines eluted with hexane. The eluent was further cleaned up and the CBs and OCPs separated using an alumina column (3 g) deactivated to 5% H_2O, followed by a silica column (3 g) deactivated to 3% H_2O (Wells and Johnston, 1977; Wells et al., 1985). The CB fraction was then transferred to iso-octane and the internal standards (D6/D16) were added.

Final determination of CBs and OCPs was carried out using a Varian 3500 gas chromatograph fitted with an electron capture detector (GC–ECD). The analysis used a CPSil 8 and CPSil 19 column (50 m $\times$ 0.25 mm) for CBs and OCPs, respectively. GC conditions and quality assurance protocols have been published previously (Megginson et al., 1994).

Contaminant concentrations below the limit(s) of quantitation (LOQ) are presented as less-than values. The LOQ was the analyte concentration in the sample giving rise to a chromatographic peak of the same height as the lowest standard and was, therefore, sample specific.

All solvents used for the chemical analyses were of the highest purity and were obtained as glass distilled grade from Rathburn Chemicals (Walkerburn, Scotland). The individual pure solid CBs were obtained from the Community Bureau of Reference (CBs 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170, 180), Promochem GmBH (CBs 31, 44, 70, 74, 128, 157, 158, 194) and Ultra Scientific (CBs 49, 110, 187). The CB numbering system follows that proposed by Ballschmiter and Zell (1980).

A number of samples were analysed using a Hewlett Packard 5989A mass spectrometer engine equipped with a HP 5890 gas chromatograph (GC–MS). Samples were manually injected onto a CPSil 8CB column using helium as a carrier gas and the analysis was carried out under electron impact (EI) ionisation.

3. Results and discussion

Sample information is given in Table 1. The concentrations of organic contaminants measured in marine turtle adipose and liver tissues are given in Table 2 and Table 3, respectively. Data from hatchlings and eggs are given in Table 4. The ΣCB and p,p′-DDE data in all tissues analysed are shown graphically in Fig. 1(a) and (b).

ΣCB concentrations were highest in the loggerhead turtles for all tissues measured. Concentrations in adipose tissue ranged from 775 to 893 μg/kg wet wt, considerably higher than observed in green turtles from the Mediterranean and leatherback turtles washed ashore on the Scottish coast, which had ranges of 39–261 and 47–178 μg/kg, respectively (Table 3). The same conclusion is drawn when the data are presented as lipid weight concentrations although the within-species variability increases. In all species, adipose tissue contained the highest organochlorine concentrations followed by liver, kidney and pectoral muscle.

As previously observed by Lake et al. (1994), the predominant congeners in all species were CBs 138, 153, 180 and 187. These compounds are found in higher proportions in industrial CB formulations and are not susceptible to metabolic degradation as they have no vicinal H atoms, a requirement necessary to allow oxidative metabolism (Boon et al., 1994). There was little difference in the proportional concentrations of CBs in different organs of the same animal.

Analysis of DDT and its isomers in the turtle samples showed that p,p′-DDE was present in the greatest concentrations, making up $>95\%$ of total DDT in the majority of samples. The observed DDT component pattern in most samples was p,p′-DDE$\gg$p,p′DDT > p,p′-DDD. This pattern is similar to that observed in most top predators at a distance from DDT point source contamination (Aguilar, 1987). The p,p′-DDE component of ΣDDT in turtle tissues was proportionately higher than for other marine species previously analysed, in agreement with data from the

Table 1
Turtle samples analysed for chlorobiphenyls and organochlorine pesticides

Sample ID	Species	Area	Sampling date	Life stage	CCL (cm)	CCW (cm)
G1	Green	Cyrprus	21/06/95	J	33	26
G2[a]	Green	Cyrprus	16/08/95	J	57	55
G3	Green	Cyrprus	22/07/95	J	35	35
G4	Green	Cyrprus	20/06/96	J	33	31
G5	Green	Cyrprus	18/07/96	J	37	32
G6[a]	Green	Cyrprus	05/09/96	J	31	28
G7	Green	Cyrprus	01/07/96	J	35	30
G8	Green	Cyrprus	15/09/96	J	32	28
G9	Green	Cyrprus	11/08/95	J	30	28
LB1	Leatherback	UK	18/10/93	M	151	nm
LB2	Leatherback	UK	24/10/95	M	141	89
L1	Loggerhead	Cyrprus	07/06/95	M	67	65
L2	Loggerhead	Cyrprus	06/09/94	J	49	47
L3[a]	Loggerhead	Greece	23/12/95	F	81	74
L4	Loggerhead	Cyrprus	02/08/95	J	23	22
L5	Loggerhead	Greece	22/12/95	J	48	43
L6	Loggerhead	UK	07/11/95	J	21	19
GH1	Green	Cyrprus	03/08/95	H	nm	nm
GH4	Green	Cyrprus	01/09/95	H	nm	nm
GH5	Green	Cyrprus	27/09/95	H	nm	nm
LH-1	Loggerhead	Cyrprus	29/07/95	H	nm	nm
LH-2	Loggerhead	Cyrprus	31/07/95	H	nm	nm
LH5	Loggerhead	Cyrprus	22/08/95	H	nm	nm
LE1	Loggerhead	Cyrprus	29/08/95	E	na	na
GE5	Green	Cyprus	14/08/95	E	na	na

J = Unsexed juvenile; M = adult male; F = adult female; H = hatchling; E = egg; CCL = curved carapace length; CCW = curved carapace width; nm = not measured; na = not applicable.

[a] Samples analysed in duplicate.

east coast of the USA for loggerhead and Kemp's ridley turtles (Lake et al., 1994; Rybitski et al., 1995). As observed for the CBs, the highest p,p′-DDE concentrations were measured in loggerhead adipose tissue.

The relationship between the curved carapace length (CCL; cm) and the total CB and p,p′-DDE concentrations measured in green turtle livers are given in Fig. 2(a) and (b), respectively. The decrease of these contaminants with increasing size was tested using Spearman's rank correlation and found to be statistically significant ($r = 0.44$, $p < 0.05$ for ΣCB; $r = 0.84$, $p < 0.001$ for p,p′-DDE). Since size is likely to be proportional to age, these results also correlated with the available knowledge of the green turtle life history. It is highly likely that the smallest of the green turtles are recent recruits into the juvenile developmental habitat (Musick and Limpus, 1997). Inspection of gut contents (Godley, unpublished data) has found that all stranded green turtles were feeding only on sea grass immediately prior to death. Prior to this stage they are likely to have passed through the pelagic omnivorous phase when they would feed at a higher trophic level. The main OC burden would be accumulated by green turtles in the pelagic stage and subsequently diluted

Table 2
Chlorobiphenyl organochlorine pesticide concentrations in turtle adipose tissue (μg/kg wet wt)

Sample ID	Extractable lipid (%)	CB28	CB52	CB101	CB118	CB153	CB138	CB180	ΣCB	Endrin	Dieldrin	p,p′-DDD	p,p′-DDE	p,p′-DDT	ΣDDT	ΣChlor
L1	51	< 5.4	24	28	71	229	132	73	775	< 6.0	9.2	< 6.0	705	8.0	739	12.0
L2	21	< 8.0	37	32	54	232	133	154	893	< 2.0	6.2	6.6	376	< 2.0	391	14
L3	73	< 4.8	8.6	8	62	261	169	116	853	10	< 1.8	< 1.8	446	4.6	454	33
LB1	74	< 7.2	12	< 7.2	< 7.2	7.8	< 7.2	< 7.2	47	< 8.1	19.0	< 8.1	10	< 8.1	14	22
LB2	50	< 4.7	12	5.8	8.3	46	25	24	178	< 6.2	13.0	< 6.2	57	< 6.2	58	12.0
G1	33	< 3.2	25	12	3.7	14	8.2	7.8	109	< 3.2	3.5	< 3.2	19	< 3.2	23	< 3.2
G2	26	< 2.2	13	5.7	< 2.2	< 2.2	< 2.2	< 2.2	39	< 1.9	< 1.9	< 1.9	2.4	< 1.9	3.3	< 1.9
G3	32	< 3.7	19	33	27	32	28	13.0	261	< 3.0	< 3.0	< 3.0	6.0	< 3.0	11	< 3.0

CB = Chlorobiphenyl; ΣCB = sum of CBs 31, 28, 52, 49, 44, 74, 70, 101, 110, 149, 118, 153, 105, 138, 158, 187, 128, 156, 157, 180, 170, 189, 194; ΣDDT = o,p′-DDD + o,p′-DDE + o,p′-DDT + p,p′-DDD + p,p′-DDE + p,p′-DDT; ΣChlor = heptachlor + heptachlor epoxide + α-chlordane + γ-chlordane + oxychlordane + *trans*-nonachlor.

Table 3
Chlorobiphenyl and organochlorine pesticide concentrations in turtle liver tissue (μg/kg wet wt)

Sample ID	Extractable lipid (%)	CB28	CB52	CB101	CB118	CB153	CB138	CB180	ΣCB	Endrin	Dieldrin	p,p′-DDD	p,p′-DDE	p,p′-DDT	ΣDDT	ΣChlor
L1	12	< 1.6	< 1.6	3.1	8.7	29	24	11	102	nm	nm	nm	76	nm	77	nm
L2	10	< 0.5	< 0.5	3.4	4.9	27	20	18	101	< 0.5	2.7	< 0.5	49	< 0.5	49	2.1
L4	3.9	< 0.5	1.3	1.5	3.3	13	12	7.3	50	< 0.2	0.3	< 0.2	68	0.7	69	1.8
L5	9.5	< 0.5	0.8	< 0.5	6.4	26	18	11	83	1.0	2.5	< 0.5	49	< 0.5	51	5.0
L6	6.8	< 0.9	15	16	12	24	20	12	159	nm	nm	nm	149	nm	152	nm
LB1	14	< 0.8	< 0.8	< 0.8	< 0.8	1.3	0.8	< 0.8	3.7	1.1	2.5	< 0.7	1.7	< 0.7	9.1	2.3
LB2	11	< 0.6	3.9	< 0.6	< 0.6	1.5	< 0.6	< 0.6	3.1	1.3	3.1	< 0.6	6.5	< 0.6	14	2.3
G1	14	< 1.5	2.2	4.1	2.8	6.1	6.0	2.5	35	0.6	3.0	< 0.7	6.2	0.8	10	< 0.7
G2	na	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.1	< 1.0	0.5	< 1.0	< 1.0	< 1.0	1.2	< 1.0
G3	16	< 1.0	2.2	3.4	3.0	9.2	7.6	5.3	45	nm	nm	nm	2.7	nm	2.7	nm
G4	7.4	< 0.8	1.0	1.3	1.3	5.8	6.4	3.5	25	< 0.4	2.4	< 0.4	1.3	< 0.4	2.2	< 0.4
G5	15	< 0.7	0.7	0.9	< 0.7	2.0	2.0	1.1	10	nm	nm	nm	1.0	nm	1.0	2.7
G6	31	< 3.4	4.3	7.4	4.8	13	11	6.7	77	< 1.0	< 1.0	< 1.0	21	< 1.0	23	< 1.0
G7	15	< 1.7	3.5	3.9	1.7	3.4	2.2	1.9	29	< 0.8	1.5	< 0.8	1.3	< 0.8	2.9	< 0.8
G8	25	< 2.4	3.0	2.8	< 2.4	12	10	7.4	47	< 1.2	1.9	< 1.2	4.2	< 1.2	5.1	3.7
G9	7.9	< 0.86	2.0	3.2	2.6	6.3	5.2	2.8	34	< 0.4	< 0.4	< 0.4	5.8	< 0.4	6.2	< 0.4

CB = Chlorobiphenyl; ΣCB = sum of CBs 31, 28, 52, 49, 44, 74, 70, 101, 110, 149, 118, 153, 105, 138, 158, 187, 128, 156, 157, 180, 170, 189, 194; ΣDDT = o,p′-DDD + o,p′-DDE + o,p′-DDT + p,p′-DDD + p,p′-DDE + p,p′-DDT; ΣChlor = heptachlor + heptachlor epoxide + α-chlordane + γ-chlordane + oxychlordane + *trans*-nonachlor; nm = not measured; na = not applicable.

Table 4
Chlorobiphenyl and organochlorine pesticide concentrations in turtle hatchlings and eggs (μg/kg wet wt)

Sample ID	Extractable lipid (%)	CB28	CB52	CB101	CB118	CB153	CB138	CB180	ΣCB	Endrin	Dieldrin	p,p′-DDD	p,p′-DDE	p,p′-DDT	ΣDDT	ΣChlor
LH1	8.4	1.0	7.3	4.0	1.5	3.2	1.8	4.1	45	0.4	1.3	1.0	104	1.9	113	3.3
LH2	8.6	0.5	3.8	2.4	2.2	3.3	2.1	0.5	22	1.3	9.2	<0.4	18	0.8	22	7.9
LH3	7.0	0.7	5.6	5.1	6.5	19	9.2	5.3	71	0.4	1.3	<0.4	49	0.4	51	2.6
LH5	4.8	0.6	4.6	3.4	1.1	2.8	1.3	0.4	23	<0.2	<0.2	<0.2	4.5	<0.2	5.3	0.9
GH1	8.1	0.6	4.0	1.9	<0.4	1.1	0.4	<0.4	13	<0.4	0.3	<0.4	0.5	3.4	5.8	0.4
GH4	7.0	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	0.2	<0.4
GH5	7.5	<0.4	0.5	<0.4	<0.4	<0.4	<0.4	<0.4	1.1	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
LE1	6.0	0.4	2.7	1.2	7.2	29	14	12	89	<0.3	0.6	0.3	154	0.4	155	1.8
GE5	7.0	0.3	2.2	1.1	<0.3	<0.3	<0.3	<0.3	6.1	<0.3	<0.3	<0.3	2.3	0.5	4.3	<0.3

CB = Chlorobiphenyl; ΣCB = sum of CBs 31, 28, 52, 49, 44, 74, 70, 101, 110, 149, 118, 153, 105, 138, 158, 187, 128, 156, 157, 180, 170, 189, 194; ΣDDT = o,p′-DDD + o,p′-DDE + o,p′DDT + p,p′-DDD + p,p′-DDE + p,p′-DDT; ΣChlor = heptachlor + heptachlor epoxide + α-chlordane + γ-chlordane + oxychlordane + *trans*-nonachlor.

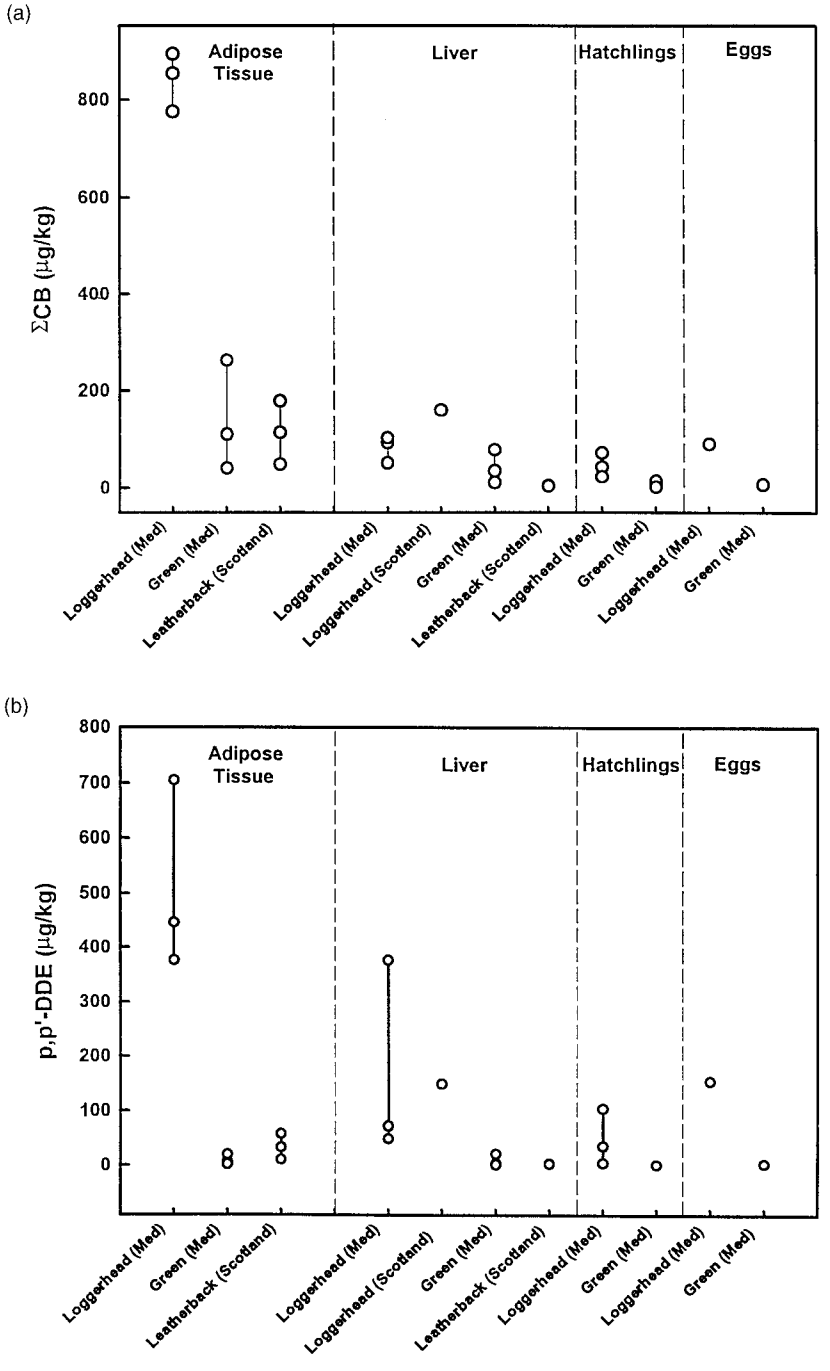

Fig. 1. Range and median of (a) ΣCB (µg/kg wet wt) and (b) p,p′-DDE (µg/kg wet wt) in marine turtle tissues, eggs and hatchlings.

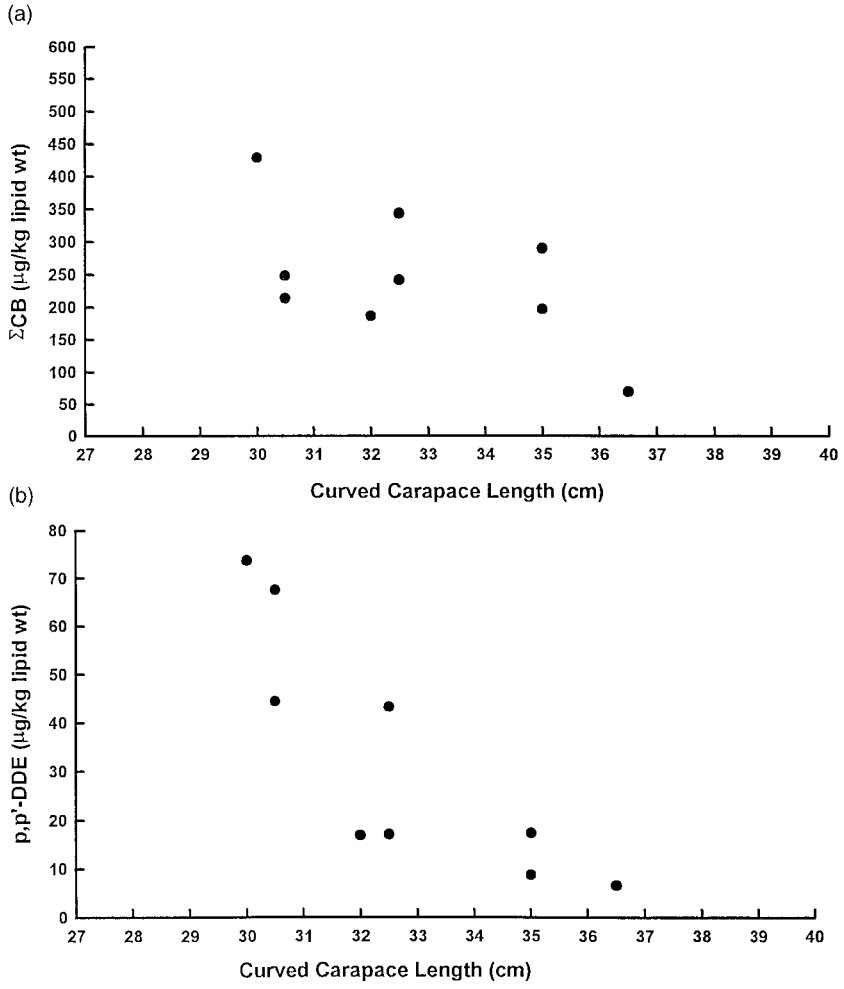

Fig. 2. Curved carapace length (CCL; cm) vs (a) ΣCB (µg/kg wet wt) and (b) p,p′-DDE (µg/kg wet wt) in green turtles.

as the animal grows during the herbivorous stage. This finding is different from observations made in the omnivorous, freshwater snapping turtle in which muscle contaminant concentrations were found to be correlated with length. The more lipophilic CB congeners were found to be highly correlated with age (Hebert et al., 1993).

There were insufficient data to study any potential bioaccumulation of OCs in loggerhead and leatherback turtles.

The insecticide dieldrin was detected in most of the tissues analysed, and was often present as the second most abundant OCP identified in the chromatogram, although at considerably lower concentrations than p,p′-DDE. The concentration ranges for the loggerhead and leatherback turtles were similar in both adipose and liver samples, while the range observed for the green turtles was lower.

Other pesticides detected were the main constituents of technical chlordane; oxychlordane, heptachlor epoxide and *trans*-nonachlor. Heptachlor, α-chlordane and γ-chlordane were not detected in any samples. The concentrations of chlordanes in the marine turtles were in the order: loggerhead > leatherback > green turtle. Chlordane residues were not detected in the three green turtle adipose samples but were detected in two of the liver samples, G5 and G8.

Lindane (hexachlorochyclohexane, γ-HCH) was detected in two of the loggerhead turtle samples at concentrations < 1 μg/kg. Hexachlorobenzene and α-HCH (an isomer of lindane) were not detected in any of the samples.

The concentrations of organic contaminants in loggerhead and green turtle hatchlings and eggs suggest a similar trend, with CB and OCP levels in loggerhead turtles being higher than in green turtles. The loggerhead turtle egg, LE1, had the second highest concentration of ΣDDT in the data set when expressed on a lipid weight basis and concentrations in the loggerhead hatchlings were similar to those of adults when expressed on a lipid weight basis.

Comparative data for ΣCB and p,p′-DDE concentrations in loggerhead, green and leatherback turtles are given in Table 5. Concentration differences between loggerhead and green turtles have been noted previously, loggerhead turtles having consistently higher concentrations in muscle, liver and eggs than green turtles (Hillestad et al., 1974; Thompson et al., 1974; Clark and Krynitsky, 1980; McKim and Johnston, 1983). The concentrations of OCs measured in loggerhead and green turtles from the Mediterranean lie within the range of those measured by others.

The largest peaks observed in the GC–ECD chromatograms of the loggerhead and green turtle CB fractions were not identified as CBs or OCPs. Chromatograms for the livers of a loggerhead turtle (L1) and a green turtle (G1) are given in Fig. 3(a) and (b), respectively, with the unknown peaks labelled A–C. The samples were run on GC–MS in EI mode; however, only one of the unknown components, peak C in the GC–ECD chromatogram, was identified using GC–MS. The total ion chromatogram and the mass spectrum for peak C are given in Fig. 3(c) and (d). The mass spectrum for peak C gave a good match ($> 95\%$) for elemental sulphur, S_8. The sulphur concentrations were not quantified but on the basis of peak size the order of tissue retention of sulphur was: liver > kidney > adipose > muscle > hatchlings = eggs.

The observed sulphur is likely to be of dietary origin as both species of turtle, especially green turtles, are known to feed at least in part on sea grasses. Additionally, the CB fraction of the leatherback turtle samples, thought to be feeding on jellyfish and other pelagic items, did not contain peaks A–C. Under anaerobic conditions, such as those in the stomach, sea grasses will decompose via sulphate reduction to H_2S. H_2S will then undergo microbial oxidation to S_8 or SO_4^{2-} (Fenchel, 1970).

Chlorinated organics enter marine turtles through their diet and through reproductive transfer from females to eggs. The degree of bioaccumulation of these contaminants depends on the trophic position of a turtle species in the marine food web, its habitat and the geographical location of individuals.

For example, in the Mediterranean, loggerhead turtles, which although omnivorous are mainly carnivorous, have higher concentrations of CBs and OCPs than

Table 5
Comparative ΣCB and p,p′-DDE studies in loggerhead, green and leatherback turtles

Species	*n*	Location	Tissue	ΣCB (μg/kg)	p,p′-DDE (μg/kg)	Reference
Loggerhead	19	Virginia/North Carolina, USA	Adipose	55–1730	3.0–1210	Rybitski et al. (1995)
	3	Cyprus/Greece	Adipose	775–893	391–739	This study
	17	Virginia/North Carolina, USA	Liver	8.3–514	< 2.0–458	Rybitski et al. (1995)
	8	Florida, USA	Liver	< 5.0–133	< 0.1–51	McKim and Johnston (1983)
	4	Cyprus/Greece	Liver	50–102	49–77	This study
	1	Scotland, UK	Liver	159	152	This study
	9	Florida, USA	Muscle	8.0	< 1.0–40	McKim and Johnston (1983)
	1	Virginia, USA	Muscle	< 2.0	< 2.0	Rybitski et al. (1995)
	1	Cyprus	Muscle	49	23	This study
	1	Virginia, USA	Kidney	4.8	< 2.0	Rybitski et al. (1995)
	1	Cyprus	Kidney	26	9.5	This study
	9	Florida, USA	Eggs	78[a]	47[c]	Clark and Krynitsky (1980)
	56	Florida, USA	Eggs	nm	99[c]	Clark and Krynitsky (1985)
	na	Georgia/South Carolina, USA	Eggs	nm	58–305[d]	Hillestad et al. (1974)
	1	Cyprus	Egg	89	155	This study
Green	3	Cyprus	Adipose	39–261	2.4–19	This study
	4	Florida, USA	Liver	< 5.0–70	< 1.0	McKim and Johnston (1983)
	9	Cyprus	Liver	< 1.1–47	< 1.0–21	This study
	2	Florida, USA	Eggs	< 25[a]	< 5.0–42	Clark and Krynitsky (1980)
	10	Ascension Island	Eggs	8[b]	nd–9.0	Thompson et al. (1974)
	1	Cyprus	Egg	6.1	4.3	This study
Leatherback	2	Scotland, UK	Liver	47–178	10–57[d]	This study
	1	England, UK	Liver	230	68[d]	Godley et al. (1998)

CB = Chlorobiphenyls; na = not available; nm = not measured; nd = not detected.
[a] Arochlor 1260 equivalents.
[b] Arochlor 1254 equivalents.
[c] p,p′-DDE + p,p′-DDT.
[d] p,p′-DDE + p,p′-DDT + p,p′-DDD.

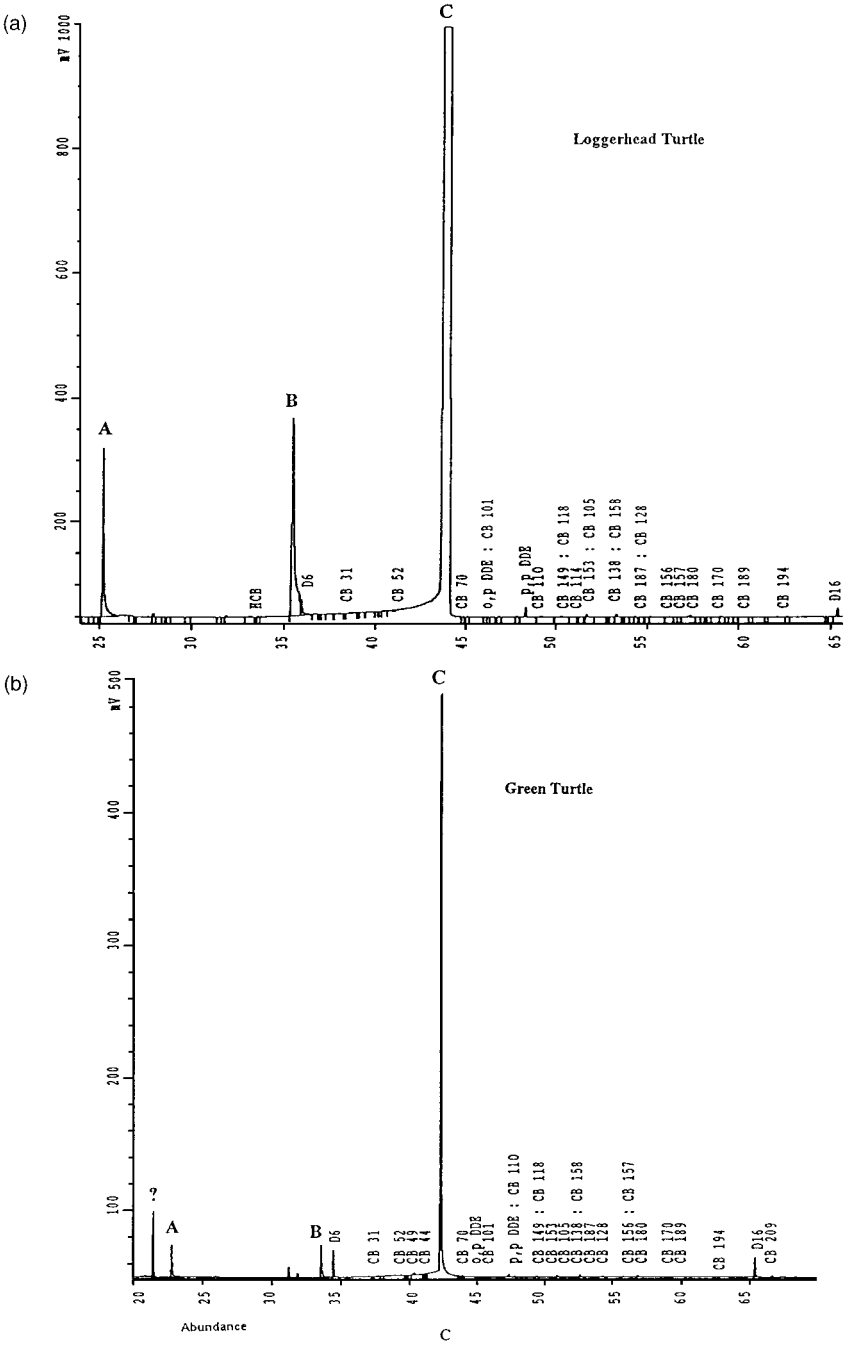

Fig. 3. *(continued on next page)*

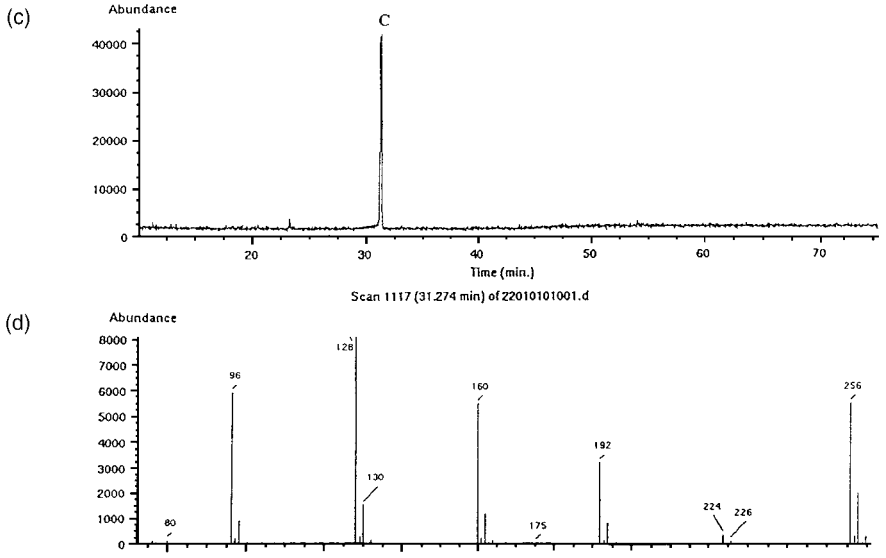

Fig. 3. GC–ECD chromatograms of (a) a loggerhead turtle liver, CB fraction; (b) a green turtle liver, CB fraction; GC–MS of (c) total of ion chromatogram and (d) mass spectrum of peak C detected in a loggerhead turtle liver, CB fraction; peak A = unknown; peak B = unknown; peak C = elemental sulphur (S_8).

green turtles which are omnivorous after hatching but subsequently herbivorous as they mature. In the turtles from the Atlantic (one loggerhead [L6]; two leatherbacks [LB1, LB2]) it was the loggerhead turtle which had the higher OC concentrations. Leatherback turtles feed almost exclusively on pelagic jellyfish which possibly explains the low exposure to OC contamination measured in this species. Although firm conclusions should not be drawn on the basis of such a small sample size, this difference in OC level may reflect a difference in diet between the two species.

The data for hatchlings and eggs (Table 4) further suggest that loggerhead turtles are exposed to relatively higher concentrations (on a lipid weight basis) of OCs, particularly p,p′-DDE, during early developmental stages. There are no published studies correlating OC concentrations with any adverse effects in marine turtles. However, at concentrations considerably higher than those observed in this study an increase in deformities and decreased hatching success rate in the freshwater snapping turtle were observed (Bishop et al., 1994).

3.1. OC pattern analysis

The differences in the pattern of OCs in each species can be masked by differences in absolute concentrations both within and between turtle species. Much of this variance may be reduced by studying the ratios of each compound to the recalcitrant congener CB153 (Boon et al., 1994). In this study those congeners which were

commonly above the LOQ in most samples and p,p′-DDE were normalised to CB153 to give contaminant patterns. Only samples which had OC concentrations above the LOQs for the selected compounds were normalised.

Principal Components Analyses (PCA) was used to study the CB and p,p′-DDE patterns in the turtle samples. When all data were included the loggerhead hatchling, LH1, was clearly separated from the other samples on the basis of the higher chlorinated CBs (CBs 170, 180, 194) and p,p′-DDE. This sample also had the highest absolute concentrations of ΣCB and ΣDDT of the four loggerhead hatchlings analysed. These hatchlings would have obtained most of the OC burden directly from their mother, but it is not immediately apparent why the high concentration should also produce a change in OC pattern. To examine the other data more closely LH1 was removed from the data set and the PCA recalculated. The results are given in Fig. 4.

Three groups have been identified in the biplot: (1) loggerhead turtle adipose tissue, liver, kidney, muscle and egg; (2) loggerhead turtle hatchlings; and (3) all tissues from green turtles. The lines drawn in the PCA biplot to visualise these groups do not have statistical significance. The green turtle samples are relatively scattered in the plot which may reflect an increase in analytical and sample variance as a result of the low OC concentrations observed, as indicated by the positions of the sample duplicates (GL5A, GL5B). In one case, samples of adipose tissue and liver (GA1, GL1) from the same animal have similar OC patterns indicating little differences between some tissues. However, the differences in patterns between the same tissues in a second animal (GA2, GL2) would appear to contradict this. In the loggerhead turtle samples there is little pattern difference between organs from the same animal (adipose tissue, liver, kidney and muscle from L1 and adipose tissue and liver from L2).

The loggerhead turtle hatchlings have higher concentrations of the lower chlorinated CBs and p,p′-DDE than the other tissues. To evaluate the differences between the hatchlings and the other samples more clearly the green turtle samples were

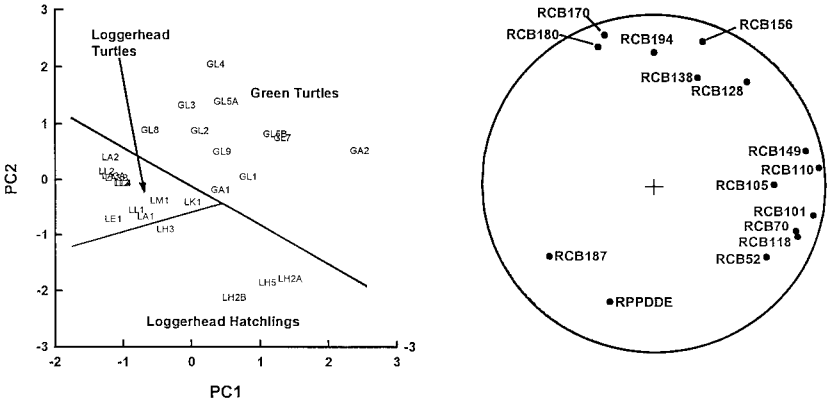

Fig. 4. Principal component analysis biplot of green and loggerhead turtle data normalised to CB153, excluding loggerhead turtle hatchling, LH1.

removed and the PCA recalculated with the loggerhead samples alone. The resulting biplot (Fig. 5) shows that, despite the degree of scatter, the three hatchlings have higher scores on PC1 than the other samples. The main conclusions to be drawn from the biplot are that hatchlings have higher relative concentrations of lower chlorinated CBs (52, 70, 101, 105, 110, 118) and lower relative concentrations of higher chlorinated CBs (CBs 156, 170, 180, 194) compared with adults of the same species. This indicates that there is a preferential transfer of lower chlorinated, less lipophilic OC components from the mother to hatchling. It is not clear why this same difference is not observed with the egg sample. This preferential reproductive transfer has been previously observed in pinnipeds and cetaceans (Green et al., 1996; Mckenzie et al., 1997) and is based on the lipophilicity (log K_{ow}) of the compound being transferred. To show this the score of each compound on PC1 was plotted against the log K_{ow} in Fig. 6. There is a significant negative correlation (Pearson coefficient, $r = 0.87$, $p < 0.001$) between an individual compound's score on PC1 in Fig. 5 and its log K_{ow}.

4. Conclusions

This study has shown that organic contaminant concentrations in three marine turtle species decreased in the order: loggerhead > green = leatherback. The OC concentrations within different tissues of the loggerhead turtles were highest in the adipose tissue, followed by liver > eggs > hatchlings > muscle > kidney. Although adipose tissue is preferable, liver and eggs are recommended for monitoring studies. This is because of the difficulty in sampling correctly and in obtaining adipose tissue in many individuals, especially juveniles, leading to sampling inconsistency which may be reflected in a higher variance in the chemical data. The differences in

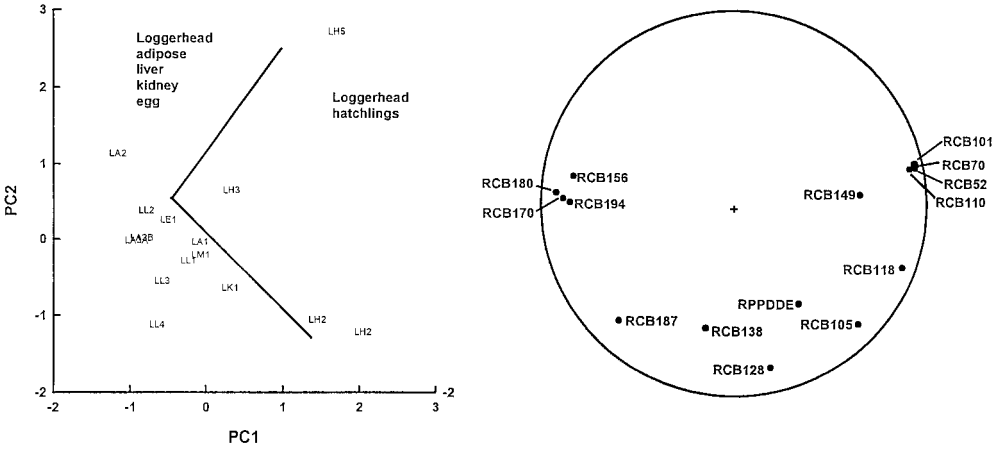

Fig. 5. Principal component analysis biplot of loggerhead turtle data normalised to CB153, excluding loggerhead turtle hatchling, LH1.

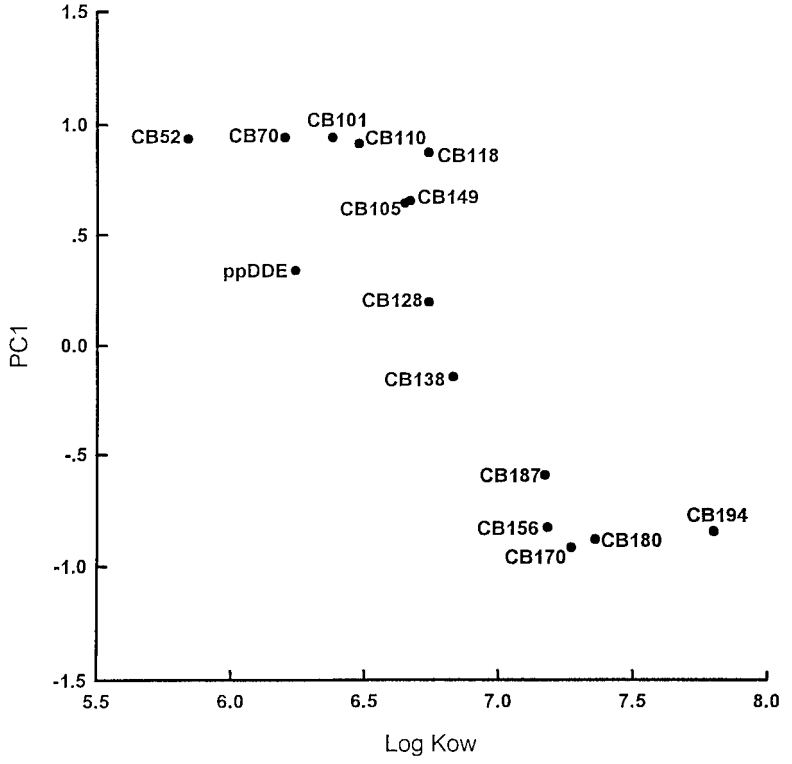

Fig. 6. Contaminant loading scores for PC1 for loggerhead turtle data normalised to CB153, excluding loggerhead turtle hatchling, LH1, vs log K_{ow}.

contaminant concentrations between species are possibly related to diet, with the varied diet of the loggerhead turtle and its higher position in the marine food web giving rise to greater exposure to contaminants than leatherback and green turtles.

The concentrations of contaminants in marine turtles from Mediterranean and Atlantic waters measured in this study are similar to those determined in the same species elsewhere in the Atlantic. The levels in all these studies are considerably lower than the concentrations shown to cause deleterious effects in freshwater turtles. In green turtles the highest contaminant burdens are found in juvenile animals but as the individual grows and there is a decrease in contaminant intake the initial concentrations are diluted.

Contaminant patterns show differences between green turtles, loggerhead turtles and loggerhead hatchlings indicating different modes of bioaccumulation. With respect to the loggerhead turtles and hatchlings this is due to selective transfer of less lipophilic contaminants from mother to offspring. The loggerhead turtle egg, did not show higher relative concentrations of lower chlorinated CBs but no conclusions have been drawn since it was only possible to determine levels for one egg from each species.

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