Very high concentrations of DDE and toxaphene residues in crocodiles from the Ord River, Western Australia: an investigation into possible endocrine disruption†

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Organochlorine pesticide concentrations, particularly those of the DDT family and of toxaphene, were measured by gas chromatography in samples of liver and body fat taken from Australian freshwater crocodiles *Crocodylus johnstoni* at three locations along the Ord River in Western Australia. The three sampling sites were the irrigation area, downstream of the irrigation area, and well upstream of the irrigation area; the last site serving as the control. DDT and toxaphene were applied in large and known quantities to cotton grown in the Ord Irrigation Area from 1964 to 1974. Thus the residues in the crocodile tissues are representative of the situation almost thirty years after the use of DDT and toxaphene ceased in the area. Very high concentrations of *p,p′*-DDE and toxaphene were found in the lipid-rich tissues that were examined. Livers and body fat from estuarine crocodiles *Crocodylus porosus* from the downstream site were also analysed. As *p,p′*-DDE and toxaphene are both known to be disruptive of endocrine systems, a range of blood parameters, including estradiol and testesterone concentrations, were also measured for all the animals studied. The ovaries and testes of the freshwater crocodiles were also examined histologically. There were no obvious effects on blood chemistry or gonad histology of the large burden of pesticides and their metabolites carried by exposed animals, although the limited number of samples and the variability of the breeding state of the animals examined may have masked possible effects. The isolation of the area, the accurately known applications of DDT and toxaphene, and the simplicity of the drainage system make the lower Ord River a unique natural laboratory for studying the long term breakdown and effects of pesticides applied in a tropical environment.

Introduction

The Ord River irrigation area (OIA) in the north-east of Western Australia is an isolated region of intensive agriculture surrounded by unirrigated rangelands and bush that support low density cattle production (map, Fig. 1). From 1964 to 1974 cotton was grown in the OIA and during that time 435 tonnes of DDT (mainly 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane) and 412 tonnes of toxaphene (a mixture of mainly polychlorinated bornanes and camphenes) were applied to the cotton crop. The Ord River, which discharges through a highly tidal estuary into Cambridge Gulf some 40 km below the OIA, received all drainage from the cotton farms. The Ord River is rich in both resident and catadromous fish species and the river and estuary support two species of crocodile.

The endocrine disrupting properties of toxaphene and of *p,p′*-DDE (2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethene), the main persistent metabolite of DDT, have been well established[1–7] but previously reported work on crocodilian species have, apart from a few simple studies[8,9] been limited to the analysis of residue levels in eggs[10–15] and to studies of juvenile American alligators *Alligator mississippiensis*[3,4,16,17] The ecotoxicology of crocodilians has been comprehensively reviewed by Campbell.[18]

The isolation of the OIA, the simplicity of its drainage system, and the exact record of pesticide application[19] make the region a valuable natural laboratory to study the long-term rate of degradation of DDT and toxaphene, and the possible endocrine disrupting effects of pesticide residues on wildlife.

In the study reported here we have measured the concentrations of organochlorine pesticides, including *p,p′*-DDE, and toxaphene, in the visceral fat and livers of Australian freshwater crocodiles *Crocodylus johnstoni* collected in 2002 from drainage channels within the OIA, from a downstream site, and from an upstream (control) site. In addition we have measured the organochlorine pesticide residue concentrations in the saltwater crocodile *Crocodylus porosus* collected from the downstream site. Thus the concentrations of DDT and its metabolites and toxaphene congeners in the two species of

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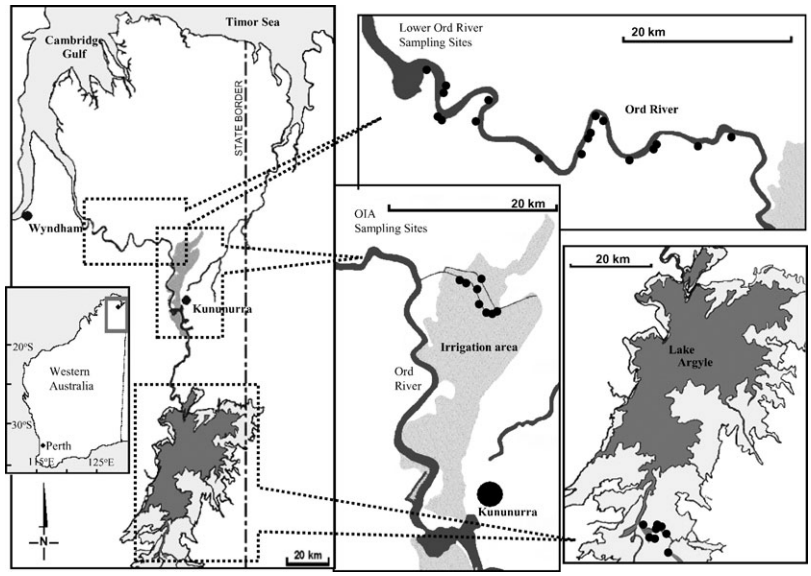

Fig. 1 Map of Ord River region showing sampling locations.

crocodile were measured 28 years after the last application of these pesticides to a cotton crop, which was the only significant source of the compounds to the habitat of the crocodiles.

To investigate the possibility that the anticipated prolonged exposure to high concentrations of, in particular, *p,p′*-DDE and toxaphene had led to some gross evidence for the disruption of the endocrine systems of the crocodiles, detailed analyses of blood serum samples were also carried out and gonad (testes or ovary) samples were taken from all animals for histological examination.

Experimental

The region and the history of pesticide application to the irrigation area

The Kimberley region of Western Australia is an area of tropical grassland and woodland and rocky mesa outcrops that has, since the time of European settlement (about 1800), been used to raise beef cattle at low densities on very large ranching properties. The region receives highly seasonal monsoonal rainfall between November and March (>80%) and, as a consequence, is drained by large rivers with river flats composed of fertile soils. The damming of the Ord River in the late 1960s and the irrigation of Ivanhoe Plains (to create the OIA) near Kununurra was the first major attempt to introduce intensive crop farming into the area. The chosen crop, cotton, was first grown in commercial quantities in 1964. Control of *Helicoverpa* spp. commonly known as the heliothis moth, a devastating cotton pest, in subsequent years required increasing applications of insecticides. Application rates became so high that the cotton-growing enterprise soon became uneconomic and the effort was abandoned with the last crop being grown in 1974.[19]

The major insecticides applied for the control of heliothis were DDT and toxaphene. The area of Ivanhoe Plains used for cotton growing increased from 650 ha in 1964 to a maximum of 4700 ha in 1967; thereafter it was reduced to about 3500 ha as the application rates for pesticides rose. Estimated totals of 435 tonnes of DDT and 412 tonnes of toxaphene were applied to the OIA in the years 1964 to 1974.[19]

The drainage system of the area is simple (Fig. 1). The Ord River, which flows overall from south to north, has been dammed in two places. The upper dam has created the massive reservoir of Lake Argyle and the second dam (the diversion dam) some 50 km downstream of the first, has created long, narrow Lake Kununurra, which supplies water, *via* a pumping station and a main irrigation channel to the farms on the OIA. In the years 1964 to 1974 these were virtually all cotton farms. All drainage from the OIA finds its way into the Ord River below the diversion dam (there is no drainage from the irrigated/sprayed area into Lake Kununurra) and then discharges through a highly tidal estuary into Cambridge Gulf.

In the study reported here we have analysed visceral fat and liver taken from Australian freshwater crocodiles *C. johnstoni* at three sites on or adjacent to the Ord River during July to October, 2002; *i.e.* 28 years since the last use of DDT and toxaphene in the area and 15 years since the use of DDT was banned. The most upstream site, south of Lake Argyle and some 130 km upstream of the sprayed irrigated farm lands served as the control area. The second collection site was the irrigation channels of the area, north of Kununurra, actually sprayed with pesticides during the years of cotton farming. The third site was downstream of the irrigated (sprayed) area and included animals taken from the tidal reach of the river. Tissues from the estuarine or saltwater crocodile *C. porosus* from the downstream site were also analysed. This species was not available from the other two sites. In addition to the pesticide analyses, blood plasma parameters were measured for each animal; these included the concentrations of 17-β-estradiol and of testosterone. Gonad samples (testes and ovaries) were taken from each animal and subjected to histological examination.

Sample collection

A total of 30 freshwater crocodiles and 10 saltwater crocodiles were collected by capture with a noose or by shooting. Use of a noose from a small boat was not possible in the drainage channels of the OIA. The small sample sizes precluded inclusion of the method of capture in the analysis of results. Animals were collected under licences TF001723 and TF001764 issued by the Western Australian Department of Conservation and Land Management. Details of the size, sex, and breeding state of the crocodiles are given in Table 1, and the locations from which they were taken are shown in Fig. 1. The approximate ages of the freshwater crocodiles were estimated from the snout-vent length (SVL)–age data of Jeffree *et al.*[20] for crocodiles from the Lynd River in north eastern Australia. Ages for the individual crocodiles is of value in assessing their exposure history, in particular to determine whether they lived through part or all of the spraying period (1964 to 1974). However, the reported double growth curves for *C. porosus*[21] makes it difficult to estimate even approximate ages for this species and it has not been attempted.

Animals collected by noosing were transported alive to laboratory facilities in damp hessian sacks. Blood samples were taken from the supra-vertebral vessel[22] and the animals were then euthanised by intra-cardiac injection of a lethal dose of sodium pentobarbitol before the livers and visceral fat were removed. Collection of live animals and removal of blood samples were performed in compliance with the guidelines of the Western Australian Department of Conservation and Land Management and with the approval of relevant Departmental authorities. Animals that were shot were processed on site and blood samples were taken from the heart. All blood

Table 1 Details of crocodiles, both *Crocodylus johnstoni* and *Crocodylus porosus*, analysed in this study

Sample	Species	Sex	Location	Total length/m	Body mass/kg	Description	SVL/mm	Estimated age/years[a]
1	*Crocodylus johnstoni*	F	Lower Ord River	1.62	13	Mature female post breeding season	815	21
2	*Crocodylus johnstoni*	F	Lower Ord River	1.28	5.3	Adolescent female post breeding season	655	13
3	*Crocodylus johnstoni*	F	Lower Ord River	1.46	8.6	Mature female post breeding season	755	18
4	*Crocodylus johnstoni*	F	Lower Ord River	1.53	8.7	Adolescent female post breeding season	765	18
5	*Crocodylus johnstoni*	M	Lower Ord River	1.77	15.8	Adolescent male post breeding season	910	27
6	*Crocodylus johnstoni*	M	Lower Ord River	2.41	47.0	Mature male post breeding season	1280	57
7	*Crocodylus johnstoni*	M	Lower Ord River	2.58	NA	Mature male post breeding season	1365	66
8	*Crocodylus johnstoni*	M	Lower Ord River	2.40	NA	Mature male post breeding season	1280	57
9	*Crocodylus johnstoni*	M	Lower Ord River	2.15	NA	Mature male post breeding season	1125	43
10	*Crocodylus johnstoni*	M	Lower Ord River	2.20	NA	Mature male post breeding season	1115	42
11	*Crocodylus johnstoni*	F	OIA	1.17	5.2	Adolescent female post breeding season	590	10
12	*Crocodylus johnstoni*	M	OIA	1.41	7.7	Adolescent male post breeding season	705	15
13	*Crocodylus johnstoni*	M	OIA	1.96	NA	Adolescent male post breeding season	1070	38
14	*Crocodylus johnstoni*	M	OIA	1.33	6.6	Juvenile male post breeding season	670	13
15	*Crocodylus johnstoni*	M	OIA	1.03	3.0	Juvenile male post breeding season	490	7
16	*Crocodylus johnstoni*	M	OIA	1.35	7.4	Juvenile male post breeding season	670	13
17	*Crocodylus johnstoni*	M	OIA	1.74	17.8	Adolescent male post breeding season	880	25
18	*Crocodylus johnstoni*	M	OIA	2.15	36.4	Mature male post breeding season	1130	43
19	*Crocodylus johnstoni*	M	OIA	1.48	13.7	Adolescent male post breeding season	840	22
20	*Crocodylus johnstoni*	M	OIA	1.57	11.6	Adolescent male post breeding season	810	21
21	*Crocodylus johnstoni*	F	Upper Ord River	1.11	3.5	Juvenile female breeding season	575	10
22	*Crocodylus johnstoni*	F	Upper Ord River	1.35	8.0	Adolescent female breeding season	705	15
23	*Crocodylus johnstoni*	F	Upper Ord River	1.67	14.7	Mature female post breeding season	850	23
24	*Crocodylus johnstoni*	F	Upper Ord River	1.40	9.2	Mature female post breeding season	720	16
25	*Crocodylus johnstoni*	M	Upper Ord River	1.24	5.5	Juvenile male post breeding season	630	12
26	*Crocodylus johnstoni*	M	Upper Ord River	1.51	9.9	Adolescent male post breeding season	760	18
27	*Crocodylus johnstoni*	M	Upper Ord River	1.44	8.8	Juvenile male post breeding season	725	16
28	*Crocodylus johnstoni*	M	Upper Ord River	1.29	7.2	Juvenile male post breeding season	665	13
29	*Crocodylus johnstoni*	M	Upper Ord River	0.92	1.7	Juvenile male post breeding season	460	6
30	*Crocodylus johnstoni*	M	Upper Ord River	1.36	7.3	Juvenile male post breeding season	695	15
31	*Crocodylus porosus*	F	Lower Ord River	1.49	7.8	Juvenile female breeding season	735	
32	*Crocodylus porosus*	F	Lower Ord River	2.11	30.0	Adolescent female post breeding season	1030	
33	*Crocodylus porosus*	F	Lower Ord River	1.99	22.7	Adolescent female breeding season	950	
34	*Crocodylus porosus*	F	Lower Ord River	2.71	68.0	Mature female breeding season	1330	
35	*Crocodylus porosus*	M	Lower Ord River	2.11	28.0	Juvenile male breeding season	1020	
36	*Crocodylus porosus*	M	Lower Ord River	3.02	115.0	Adolescent male breeding season	1510	
37	*Crocodylus porosus*	M	Lower Ord River	2.11	31.0	Juvenile male breeding season	1015	
38	*Crocodylus porosus*	M	Lower Ord River	3.35	162.0	Mature male breeding season	1700	
39	*Crocodylus porosus*	M	Lower Ord River	1.42	8.2	Juvenile male breeding season	705	
40	*Crocodylus porosus*	M	Lower Ord River	3.13	113	Adolescent male post breeding season	1560	

[a] Estimated from SVLs using data of Jeffree *et al.* (ref. 20).

samples were centrifuged immediately after collection and the plasma obtained was stored and transported in liquid nitrogen dewar vessels at −200 °C before analysis. Blood samples were not taken for pesticide residue analyses because of uncertainties about obtaining adequate amounts from all animals for reliable analytical results. Samples of liver and visceral fat were stored or transported at −20 °C or on dry ice before analysis. Ovaries were removed from females and testes from males immediately following the deaths of the animals and were stored and transported in 10% neutral-buffered formalin prior to histological examination.

Sample analysis

Organochlorine pesticide residue analyses. Compounds quantified are given in Table 2 for *C. johnstoni* and *C. porosus*. Diethyl ether 300, dichloromethane for dioxin analysis, and florisil PR were obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Cyclohexane 300, and hexane and acetone (both for dioxin analysis) were from Kanto Chemical Co. Inc., Tokyo, Japan. Anhydrous sodium sulfate was from Nacalai Tesque Inc., Kyoto, Japan. Merck KGaA, Darmstadt, Germany supplied silical gel 60, and Bio-beads S-X3 (200–400 mesh) were from Bio-Rad Laboratories, Hercules, CA, USA. Unlabelled analytical standards, including those for 22 toxaphene congeners, were supplied by Dr Ehrenstorfer GmbH, Augsburg, Germany. All glassware was heated at 410 °C overnight to clean it before use. Samples of liver (5 g) or visceral fat (1 g) were weighed into a glass beakers and each intimately mixed with an equal weight of hydromatrix (Varian, Harbor City, CA, USA). Each sample was then transferred to a cell of an accelerated solvent extraction (ASE) apparatus (Dionex, Sunnyvale, CA, USA) for pressurised extraction and extracted twice with acetone/hexane (1 : 1, v/v) at 100 °C for 10 min. The extracts were reduced to 5 mL each by rotary evaporation and dried with anhydrous sodium sulfate. Each extract was then spiked with ^{13}C-labelled internal standards for α-, β-, γ- and δ-HCH, oxychlordane, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, heptachlor, heptachlor epoxide, HCB, *o,p′*- and *p,p′*-DDT, *o,p′*- and *p,p′*-DDE, aldrin, endrin and diedrin, and deuterium-labelled *p,p′*-DDD (Cambridge Isotope Laboratories) to facilitate the determination of analytical recoveries. ^{13}C-Labelled *trans*-chlordane served as the surrogate for toxaphene. Lipids in the samples were removed by gel permeation chromatography (GPC) on Bio-beads that had been swollen with 1 : 1 cyclohexane/dichloromethane and packed into a custom-made teflon column (480 × 22 mm). Endrin and dieldrin were separated from the other analytes by column chromatography on florisil that had been activated at 200 °C for 18 h (elution with 5%, and then 20%, diethyl ether in hexane). HCB, aldrin and mirex were separated from the remaining analytes by column chromatography on silica gel (elution with hexane followed by 25% diethyl ether in hexane). All fractions for analysis were reduced to 500 μL under a stream of dry nitrogen, spiked with ^{13}C-labelled PCB 153 (2,4,5,2′,4′,5′-hexachlorobiphenyl) to enable allowance to be made for any small variations in injection syringe volume for all analytes including toxaphene, and placed in 1.5 mL glass vials.

Quantification of the analytes was carried out by GC-MS using a portable mass spectrometer 5973N mass selective detector equipped with a 6890 series gas chromatograph (both from Agilent Technologies, DE, USA) fitted with an HT8 fused silica capillary column (50 m × 0.22 mm id, 0.25 μm film thickness; SGE Japan, Kanagawa, Japan). Helium was the carrier gas at a flow rate of 1 mL min^{-1}. An aliquot (1 μL) of each final concentrated extract was injected with an autosampler 7673 (Agilent Technologies, DE, USA) using a splitless mode (pulsed splitless mode for toxaphene). The injector port and the transfer line in the GC were maintained at 260 °C (220 °C for toxaphene) and 280 °C, respectively. The column temperature program for all compounds except toxaphene was 50 °C for 0.3 min, ramped to 200 °C at 20 °C min^{-1}, to 280 °C at 2.5 °C min^{-1}. and maintained at 280 °C for 1 min. For toxaphene the program was 60 °C for 1 min, ramped to 170 °C at 23 °C min^{-1}, 7.5 min at 170 °C, then ramped to 275 °C at 3 °C min^{-1} , maintained at 275 for 12 min. Methane was used as the reagent gas for negative ion chemical ionization under a pressure of 2.4 × 10^{-5} kPa. The temperatures of the ion source and quadrupole were held at 150 °C and 106 °C, respectively. The mass spectrometer was operated in the selected ion monitoring (SIM) mode. Monitored ions are listed in Table 3.

Toxaphene: The problems inherent in the analysis of toxaphene have been described by de Geus *et al.*[7] Toxaphene is a complex mixture of chlorinated bornanes, bornenes, bornadienes, camphenes and dihydrocamphenes. For example, Jongbloed *et al.* reported[23] the presence of more than 180 congeners with 75% present as chlorinated bornanes whereas Jansson and Widequist reported[24] the separation of 670 individual components in technical toxaphene. The toxaphene congeners detected and quantified in *C. johnstoni* and *C. porosus* are shown in Table 4. We have taken 'total toxaphene' to be the sum of the congeners (or 'parlars') that were included in the standard. It is thus probable that total toxaphene is substantially underestimated in this study. A discussion of the problems resulting from the limited availability of analytical standards for toxaphene is included in the paper of de Geus *et al.*[7]

Blood serum analysis. Serum analysis was carried out by the Biochemical Laboratory at Royal Perth Hospital using the techniques routinely applied to the analysis of human serum. Analyses for sex hormones, testosterone and β-estradiol, were performed with radio isotope techniques. Those parameters investigated are listed in Table 5 for *C. johnstoni* and *C. porosus*.

Histology of gonads. Males: the status of seminiferous tubules (size, vacuolation) and of spermatogenesis, the volume of interstitial tissue, and the development of parasitic granulomas in interstitial tissue were investigated by visual examination under light microscopy.

Females: the extent of disappearance of ovarian follicles, the existence and amount of vitellus in the ovarian follicle, the existence and number of corpora albicantia in the ovaries, the extent of dilation of lymph ducts in the ovaries, the extent of hyalinisation of of blood vessel walls were investigated by visual examination under light microscopy.

Table 2 Concentrations of organochlorine pesticides and their transformation products in tissues of crocodiles *Crocodylus johnstoni* and *C. porosus* (ng g^{-1})

	Crocodylus johnstoni																								*Crocodylus porosus*							
	Lower Ord River								Ord Irrigation Area								Upper Ord River								Lower Ord River							
	Visceral fat on extractable lipid basis				Liver on extractable lipid basis				Visceral fat on extractable lipid basis				Liver on extractable lipid basis				Visceral fat on extractable lipid basis				Liver on extractable lipid basis				Visceral fat on extractable lipid basis				Liver on extractable lipid basis			
	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min
α-HCH	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
β-HCH	10	0	0	0	10	0	0	0	10	0	1	0	10	1	8	0	9	1	1	1	10	0	0	0	10	0	0	0	10	2	8	0
γ-HCH	10	0	0	0	10	1	7	0	10	0	0	0	10	0	1	0	9	0	0	0	10	0	0	0	10	0	1	0	10	0	0	0
δ-HCH	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
HCB	10	4	8	2	10	7	17	1	10	4	6	1	10	9	27	0	9	2	3	2	10	1	1	0	10	4	7	2	10	6	16	0
Heptachlor	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	3	0
cis-Heptachlor epoxide	10	3	17	0	10	102	563	0	10	3	5	0	10	73	397	6	9	0	0	0	10	0	0	0	10	1	6	0	10	19	44	0
trans-Heptachlor epoxide	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Aldrin	10	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
Endrin	10	0	0	0	8	0	0	0	10	1	1	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	9	0	0	0
Dieldrin	10	6	46	0	8	7	14	0	10	12	52	2	10	10	60	0	9	1	5	0	10	0	0	0	10	5	17	1	9	17	54	0
o,p′-DDD	1	0	0	0		0	0	0	10	0	0	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
p,p′-DDD	10	163	830	9	10	164	1291	0	10	236	420	53	10	293	1240	63	9	0	0	0	10	0	0	0	10	0	0	0	10	4	36	0
o,p′-DDE	10	0	0	0	10	0	0	0	10	6	10	1	10	5	31	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
p,p′-DDE	10	53 257	124 439	1302	10	42 217	272 056	2172	10	25 562	62 329	3985	10	251 893	672 610	28 941	9	8	21	5	10	0	0	0	10	5460	20 709	257	10	3361	14 286	71
o,p′-DDT	10	0	0	0	10	0	0	0	10	0	2	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
p,p′-DDT	10	0	0	0	10	148	1324	0	10	28	70	4	10	0	0	0	9	0	0	0	10	0	0	0	10	0	0	0	10	0	0	0
cis-Chlordane	10	0	1	0	10	1	3	0	10	1	2	0	10	1	3	0	9	0	0	0	10	0	0	0	10	0	0	0	10	1	7	0
trans-Chlordane	10	1	1	0	10	1	5	0	10	0	1	0	10	0	0	0	9	0	0	0	10	0	0	0	10	0	1	0	10	2	10	0
cis-Nonachlor	10	2	11	0	10	3	12	0	10	3	6	1	10	4	16	1	9	0	0	0	10	0	0	0	10	0	1	0	10	1	3	0
trans-Nonachlor	10	0	1	0	10	1	2	0	10	1	2	0	10	2	6	1	9	0	0	0	10	0	0	0	10	0	1	0	10	1	5	0
Oxychlordane	10	5	32	0	10	17	98	0	10	4	7	1	10	11	33	2	9	0		0	10	0	0	0	10	2	6	0	10	8	35	0
Mirex	10	3	10	0	10	7	50	0	10	0	1	0	10	1	3	0	9	1	2	0	10	0	0	0	10	2	7	0	10	5	22	0
Total toxaphene	10	28	92	2	10	45	125	2	10	325	739	48	10	311	1840	20	—	—	—	—	—	—	—	—	10	6	17	0	10	91	643	1

Statistical treatment. Student's *t* tests were used to determine significance of differences in blood parameters between pairs of locations.

Results and discussion

Analyses of organochlorine compounds

Concentrations of organochlorine compounds were determined and reported on a wet basis, and on the basis of solvent (acetone/hexane 1 : 1) extractable lipid. Linear regression of *p*,*p*′-DDE concentrations on a wet weight basis on *p*,*p*′-DDE concentrations on an extractable lipid basis for visceral fat ($r = 0.92$), and for liver indicated that visceral fat tissue consisted of about 53% extractable lipid, whereas liver contained about 1.2% extractable lipid. In this section we will consider only the results obtained on a extractable lipid basis.

A summary of the results of GC analyses of all organochlorine compounds is given in Table 2 for *C. johnstoni* and *C. porosus*, and the relationships between the concentrations of the major analytes with crocodile size as the snout-vent length (SVL) and estimated age (*C. johnstoni*), or with just SVL (*C. porosus*) are illustrated in Fig. 2 and 3. In addition to this the relationships between the concentrations of some compounds to demonstrate their co-accumulation are given in Fig. 4a–d. The relationships between the concentrations of *p*,*p*′-DDE and toxaphene in liver and visceral fat are shown in Fig. 5a and b.

Most percentage recoveries for the analytes were in the range 70 to 120% although a value of only 34% was determined for aldrin residues in liver samples form *C. johnstoni* from the upper Ord River, and values in excess of 400% were measured for *p*,*p*′-DDT residues in some fat samples from *C. johnstoni* from the lower Ord site.

All results for each tissue of each individual crocodile are given as supplementary material.†

Table 3 Monitored ions in GC-MS analysis of organochlorine pesticides and their breakdown products

Compounds	Q ion	C ion	^{13}C
Aldrin	330.00	237.00	342.00
Dieldrin, endrin	380.00	346.00	392.00
HCH (α, β, γ and δ)	255.00	71.00	261.00
HCB	284.00	250.00	290.00
Heptachlor	300.00	266.00	310.00
cis- and *trans*-heptachlor epoxide	388.00	282.00	398.00
cis- and *trans*-chlordane	410.00	374.00	420.00
cis- and *trans*-nonachlor	444.00	300.00	454.00
Oxychlordane	424.00	352.00	434.00
o,*p*′- and *p*,*p*′-DDD	248.00	320.00	256.00
o,*p*′-DDE and *o*,*p*′-DDT	246.00	281.00	258.00
p,*p*′-DDE	318.00	281.00	330.00
p,*p*′-DDT	283.00	318.00	293.00
Mirex	368.00	403.00	378.00
Hexachlorocamphenes	306.90	304.90	
Heptachlorobornanes	342.90	340.90	
Octachlorobornanes	376.90	374.90	
Nonachlorobornanes	412.80	410.80	
Decachlorobornanes	444.80	442.80	

Table 4 Concentrations of toxaphene congeners in tissues of crocodiles *Crocodylus johnstoni* and *C. porosus* (ng g^{-1})

	Crocodylus johnstoni												*Crocodylus porosus*					
	Lower Ord River				Ord Irrigation Area								Lower Ord River					
	Visceral fat on extractable lipid basis			Liver on extractable lipid basis				Visceral fat on extractable lipid basis			Liver on extractable lipid basis		Visceral fat on extractable lipid basis			Liver on extractable lipid basis		
	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min
Parlar 21	10	0	0	0	10	0	0	0	10	0	0	0	10	13	74	0	10	Nd			10	Nd		
Parlar 25	10	0	0	0	10	0	0	0	10	2	11	0	10	0	0	0	10	Nd			10	Nd		
Parlar 26	10	0	0	0	10	0	0	0	10	4	37	0	10	18	85	0	10	Nd			10	Nd		
Parlar 31	10	0	0	0	10	0	0	0	10	4	10	0	10	4	39	0	10	Nd			10	Nd		
Parlar 38	10	0	0	0	10	0	0	0	10	79	219	13	10	3	35	0	10	Nd			10	Nd		
Parlar 50	10	19	58	1	10	30	91	1	10	201	434	28	10	219	1475	15	10	5.2	14.2	0	10	82	608	1.0
Parlar 63	10	8	34	0	10	14	66	0	10	35	77	6	10	54	325	6	10	0.8	2.4	0	10	9	35	0
Total Toxaphene	10	28	92	2	10	45	125	2	10	325	739	48	10	311	1840	20	10	5.9	16.5	0	10	91	643	1.0

Nd = not detected. Parlars 11, 12, 15, 32, 39, 40, 41, 42, 44, 51, 56, 58, 59, 62 and 69 were also not detected. No toxaphene congeners were detected in samples from upper Ord crocodiles.

Table 5 Blood serum parameters for crocodiles *Crocodylus johnstoni* and *C. porosus*

	Crocodylus johnstoni												*Crocodylus porosus*			
	Lower Ord River				Ord Irrigation Area				Upper Ord River				Lower Ord River			
	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min	Samples	Mean	Max	Min
Estradiol/pmol L^{-1}	7	7	19	5	10	5	5	5	10	25	147	5	9	111	683	5
Testosterone/nmol L^{-1}	7	0.15	0.15	0.15	10	1.08	9.40	0.15	10	0.15	0.15	0.15	9	2	17	0.15
Sodium/mmol L^{-1}	6	143	158	126	10	138	143	126	10	149	153	142	9	138	159	89
Potassium/mmol L^{-1}	6	4.0	5.1	3.3	10	4.6	5.9	3.1	10	4	6.5	3	9	5	9.2	2.8
Chloride/mmol L^{-1}	6	115	135	88	10	110	116	105	10	116	123	106	9	115	123	102
CO_2/mmol L^{-1}	7	13	19	6	10	14	21	7	10	14	19	7	9	18	38	0.5
Urea/mmol L^{-1}	6	0.5	0.7	0.4	10	0.6	0.7	0.2	10	0.4	0.9	0.05	9	0	0.6	0.05
Creatinine/μmol L^{-1}	6	73	154	13	10	38	77	2	10	34	82	8	9	3	11	1
Glucose/mmol L^{-1}	6	3.3	7.5	1.7	10	5.4	9.9	2.6	10	7.7	11.4	4.9	9	6	25.5	1.6
Uric acid/mmol L^{-1}	7	0.12	0.28	0.04	10	0.08	0.15	0.05	10	0.27	0.53	0.14	9	0	0.62	0.1
Cholesterol/mmol L^{-1}	6	2.7	4.2	2.1	10	1.8	2.4	1.3	10	3.2	4.3	1.6	9	4	5.3	2.1
Triglyceride/mmol L^{-1}	6	0.4	1.8	0.05	10	0.58	1.30	0.05	10	1.0	4.1	0.2	9	1	9.5	0.05
HDL/mmol L^{-1}	6	0.54	0.97	0.04	10	0.38	0.69	0.13	10	0.45	0.87	0.14	9	1	1.73	0.05
LDL/mmol L^{-1}	6	2.0	3.3	1.2	10	1.2	1.6	0.8	10	2.3	3.7	1	9	2	4.6	0.4

DDT compounds and toxaphene in crocodile tissues

Very high concentrations of *p,p′*-DDE and of total toxaphene were found in both visceral fat and livers of freshwater crocodiles from the irrigation channels of the OIA and the downstream sampling locations and these were correlated with the estimated ages of the animals (Table 2, Figs. 2 and 3). The concentrations of *p,p′*-DDE and toxaphene are some of the highest ever reported for wildlife and the mean value for *p,p′*-DDE for the OIA in liver lipids of >250 μg g^{-1} compares with mean concentrations of about 6 μg g^{-1} in the blubber of toothed whales from the Arctic (AMAP, 2002) and 10 μg g^{-1} in liver lipids of the glaucous gull, also from the Arctic (AMAP, 2002). For toxaphene, the mean concentration of about 0.3 μg g^{-1} (maximum about 2μg g^{-1}) for liver lipids for freshwater crocodiles from the OIA compare with up to 30 μg g^{-1} in the lipids of lake trout from the Great Lakes of North America,[25] and >80 μg g^{-1} in the blubber of white-beaked dolphins from the coast of Newfoundland.[26] In a global context *p,p′*-DDE and toxaphene, like other persistent organochlorine compounds, have tended to move towards the poles with air and water currents from mid-latitude and tropical sources, and subsequent biomagnification in Arctic food webs, in particular, has occurred.[27,28] However, extremely high concentrations of *p,p′*-DDE in particular, have been recorded near point sources of pollution when the pesticides were being manufactured and used. For example, the blubber of male California sea lions stranded in California in 1970 contained a mean of 0.1% total DDT on a wet weight basis![29] Presumably this value would be about twice as high on an extractable lipid basis. The source of the contamination was the world's largest DDT manufacturer, which from 1948 to 1970 discharged up to 20 tons of DDT wastes annually into the Los Angeles outfall on the Palo Verdes continental shelf in southern California. When DDT in California sea lion blubber was analysed for animals collected in 2000, the concentrations of total DDT had fallen by an order of magnitude to a mean of around 100 μg g^{-1}.[30] We have no way of knowing if a similar fall in the concentrations of *p,p′*-DDE and toxaphene has occurred in Ord River crocodiles over a similar time but it must be possible. From 1964 to 1974 an average of about 40 tonnes each of DDT and toxaphene were sprayed annually on a relatively small cultivated area and substantial amounts of the compounds would have moved into the drainage channels and into the Ord River. Remarkably high concentrations of *p,p′*-DDE and toxaphene remain in the crocodile tissues almost 30 years after the use of DDT and toxaphene in the area had ceased. The longevity of crocodiles will favour the accumulation of these persisent chemicals; the estimated ages of the oldest freshwater crocodiles[20] sampled show that they would have lived through the entire period of spraying.

Freshwater crocodiles studied in the Lynd River in northern Queensland were shown[20] to have restricted home ranges and thus the animals sampled from the Ord system were also likely to be of a relatively sedentary nature and thus would have been continually exposed to *p,p′*-DDE and toxaphene throughout their lives. However, it has recently been shown that the saltwater crocodile *C. porosus*, specifically in the Ord estuary, is probably more mobile than the freshwater crocodiles with males being more active than females.[31] Thus the animals that were sampled may have spent time in less contaminated environments and this may account for the lower levels of *p,p′*-DDE and toxaphene in their tissues. Indeed, the concentrations of these persistent chemicals may, when age is taken into consideration, be taken as an indication of the time that each individual has spent in the Ord estuary.

No DDT or toxaphene were sprayed over the downstream sampling area. Although there may have been some minimal spraydrift, it is probable that the overwhelming majority of the *p,p′*-DDE and toxaphene congeners found in the crocodiles of both species sampled in the downstream locations have arrived there by riverine transport, dissolved in water or adsorbed on sediment particles. There is also the possibility that more mobile food organisms, in particular mullet, might be responsible for some dispersion of pesticides in the way that is relevant to the accumulated concentrations in the crocodiles. It is apparent though that the concentrations of toxaphene in the freshwater crocodiles from the downstream site are considerably lower in relation to the concentrations of *p,p′*-DDE than are those from the OIA. The ratios of *p,p′*-DDE/total

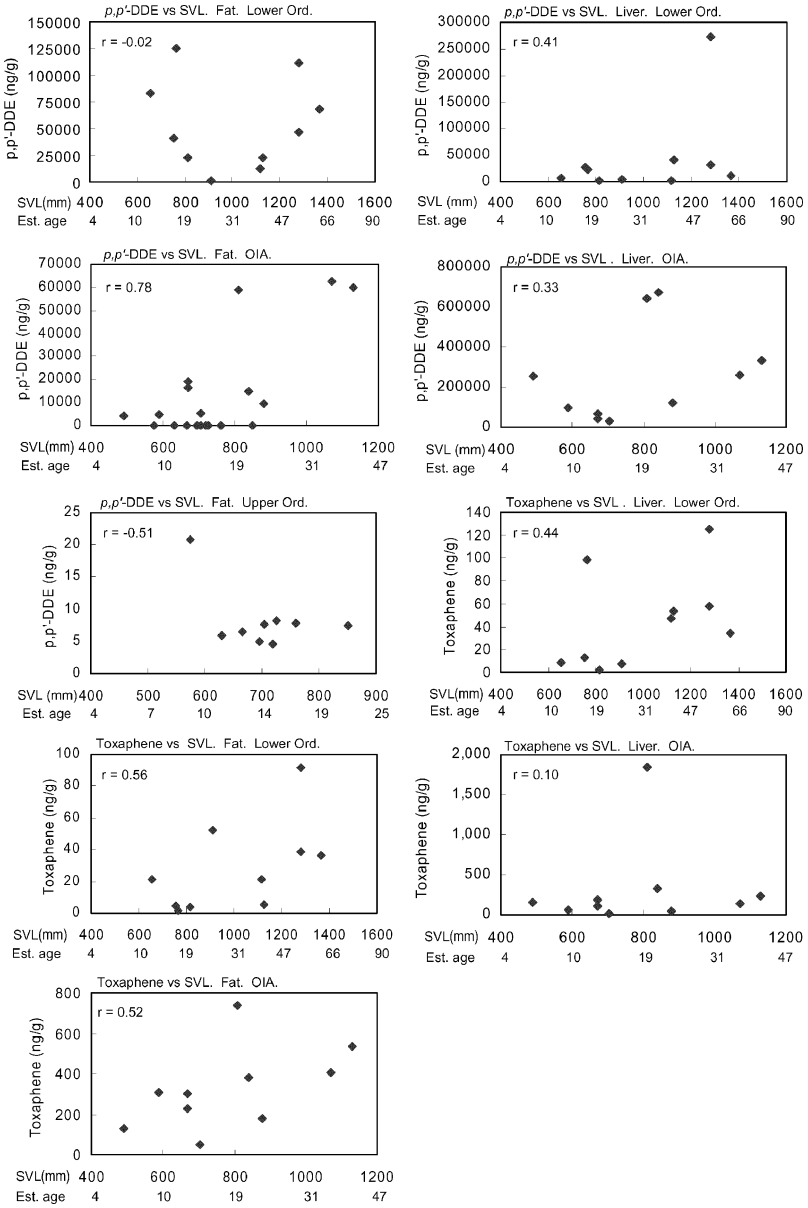

Fig. 2 *Crocodylus johnstoni*: *p,p′*-DDE and toxaphene concentrations in visceral fat and liver, both on an extractible lipid basis, *vs.* snout-vent length (SVL) for the 3 sampling locations. Toxaphene concentrations for the upper Ord River location were below the detection limit.

toxaphene for the two sampling sites are shown in Fig. 4a–d and it is obvious that the value for the lower Ord is much higher than that for the OIA. This suggests that *p,p′*-DDE is very much more mobile in the river than is toxaphene. Toxaphene is reported[7] to have a higher water solubility than *p,p′*-DDE[32] although both are low (550 μg L^{-1} at 20 °C for toxaphene; 1–40 μg L^{-1} for *p,p′*-DDE). Thus, if mobility was dependent on water solubility alone toxaphene should be more mobile than *p,p′*-DDE. This is the opposite of what was found and other factors must be important in determining the extent of riverine transport, the strength of binding to sediment particles for example. It is noteworthy that in the major studies of toxaphene in fish from the Great Lakes and in Arctic or sub-Arctic mammals, aerial transport has been the major route of contaminant movement.[25,26]

For the lower Ord sampling site, *p,p′*-DDE concentrations were generally higher in visceral fat than in liver lipids for each individual animal but the opposite was true for the OIA where liver lipids had higher conentrations than did visceral fat samples (Fig. 5a). For toxaphene, the concentrations in visceral fat and in liver lipids for each animal were similar (Fig. 5b). We are not sure why there should be such pronounced differences in the accumulation patterns for the two chemicals and the two sampling sites.

As expected, *p,p′*-DDE was the predominant DDT metabolite in the tissue samples; this reflected the presumed dominance of *p,p′*-DDT over *o,p′*-DDT in the commercial product that was applied to the cotton crop, and the accepted major breakdown pathway of DDT, by dehydrochlorination, to yield the unsaturated *p,p′*-DDE. However, the parent DDT

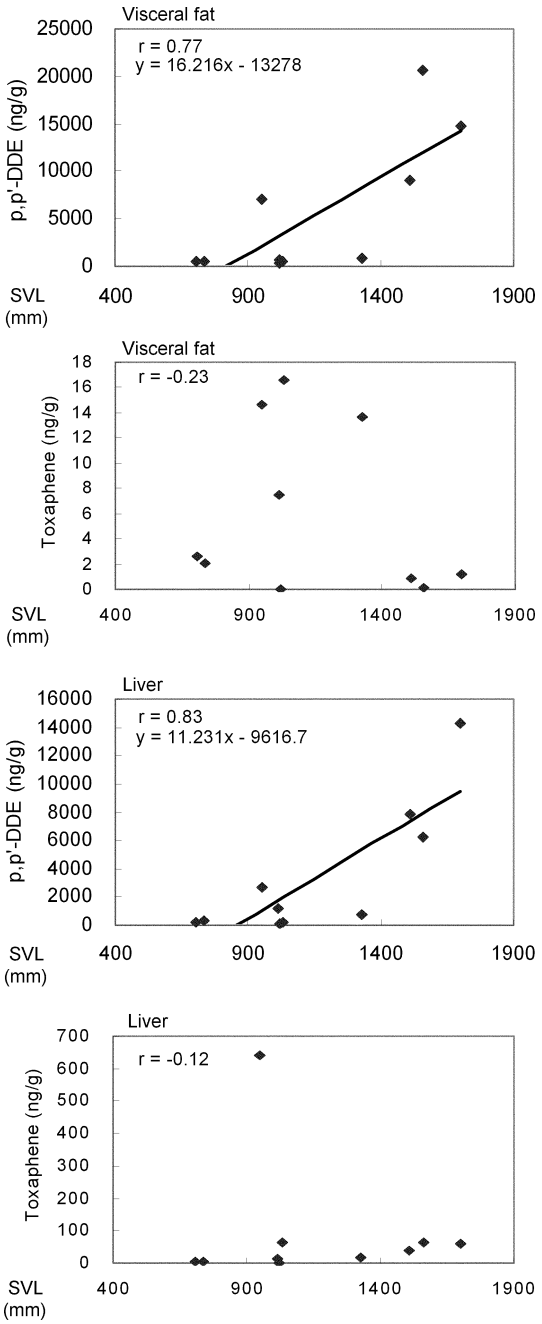

Fig. 3 *Crocodylus porosus*: *p*,*p*′-DDE and toxaphene concentrations in visceral fat and liver, both on an extractable lipid basis, *vs.* snout-vent length (SVL) for the lower Ord River sampling site.

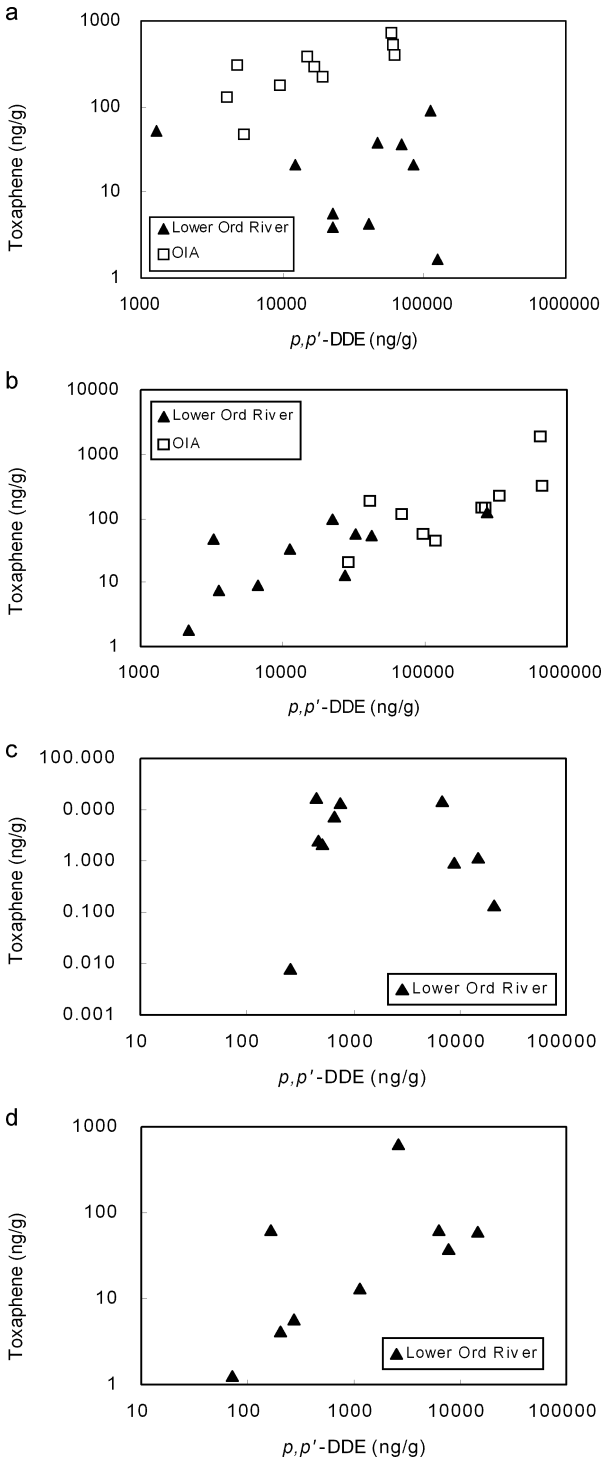

Fig. 4 (a) *Crocodylus johnstoni*: total toxaphene *vs.* *p*,*p*′-DDE concentrations in visceral fat on an extractable lipid basis. (b) *Crocodylus johnstoni*: total toxaphene *vs.* *p*,*p*′-DDE concentrations in liver on an extractable lipid basis. (c) *Crocodylus porosus*: total toxaphene *vs.* *p*,*p*′-DDE concentrations in visceral fat on an extractable lipid basis. (d) *Crocodylus porosus*: total toxaphene *vs.* *p*,*p*′-DDE concentrations in liver on an extractable lipid basis.

and its degration product formed by dechlorination, DDD, both predominantly as *p*,*p*′- substituted forms, were found in addition to the *p*,*p*′-DDE compounds in visceral fat but not in liver samples from the OIA. In contrast, *p*,*p*′-DDT was found in liver lipids from the lower Ord River and *p*,*p*′-DDD in both visceral fat and liver. The reasons for these clear-cut differences are not immediately apparent but must lie in the complexities of the metabolism and degradation of the compounds both outside and inside the animals and in the kinetics of accumulation. Linear regression of *p*,*p*′-DDE in visceral fat (extractable lipid basis) on *p*,*p*′-DDT concentration for crocodiles from the OIA ($r = 0.82$) showed that the concentration of *p*,*p*′-DDE was about 1000× that of *p*,*p*′-DDT. The observation that the concentration of *p*,*p*′-DDT is related to

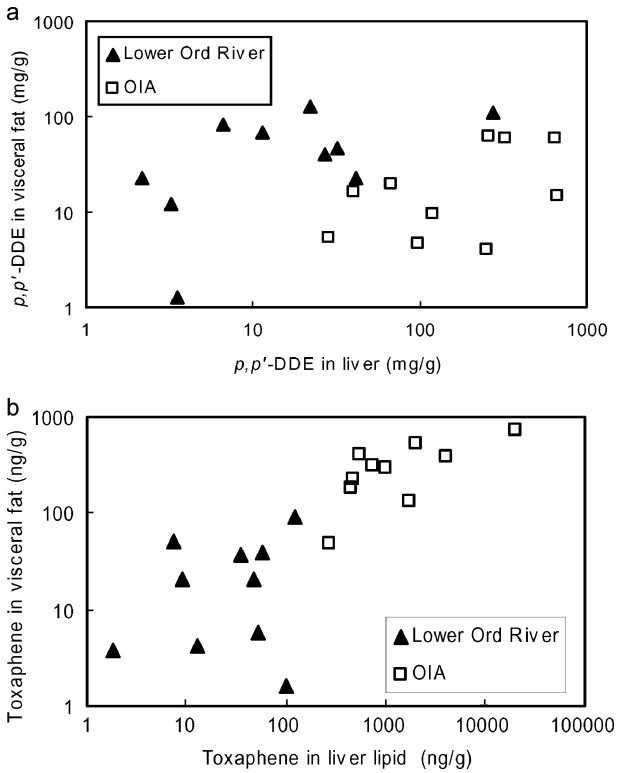

Fig. 5 (a) *Crocodylus johnstoni*: *p,p′*-DDE concentrations on an extractable lipid basis-visceral fat *vs.* liver. (b) *Crocodylus johnstoni*: total toxaphene concentrations on an extractable lipid basis-visceral fat *vs.* liver.

the estimated age of the crocodiles suggests that the DDT has been accumulated over a long period and has remained unmetabolised in the visceral fat of the animals (Fig. 6).

Toxaphene congener accumulation patterns

Previous work on the identification of toxaphene congeners in wildlife has shown the presence of parlars 26, 40, 41, 42, 44, 50, 51 and 62 in trout from Lake Superior,[33] with 50 and 62 being the major compounds. Parlars 26, 40, 41, 50 and 62 have also been identified in beluga whales[34] and five species of seals.[35] Again parlars 50 and 62 were major contributors to the total

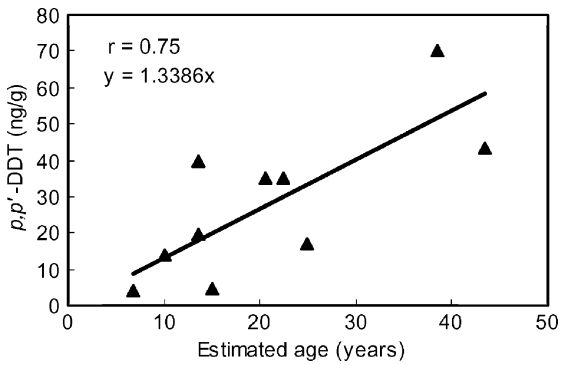

Fig. 6 *Crocodylus johnstoni*: *p,p′*-DDT concentrations in visceral fat on an extractable lipid basis *vs.* estimated age for the Ord Irrigation Area.

burden. Recently the presence of parlars 26, 50 and 62 has also been reported in white whales from Svalbard, Norway.[36]

Our results on crocodile tissues (Table 4) thus show a major difference from previous reports on environmental samples as we have identified parlar 63 as a major component of the total toxaphene burden of some samples. At the same time we were unable to identify parlar 62, which has previously been identified in trout, whale and seal tissues.[33–36] Because of this difference we were careful to confirm the identity of parlar 63. It had an identical retention time to standard material and the ratio of two different monitored masses (those used for identification and for quantification) was identical to that of the standard compound. These factors made a misidentification very unlikely. At the same time, the analysis was rather insensitive to parlar 62 and it was thus not possible to rule out its presence at trace amounts.

Previous work on the identification of toxaphene congeners in wildlife[33–36] has considered animals that were contaminated after long distance aerial transport of toxaphene. Our study is the first, to our knowledge, that has analysed the concentration of a range of toxaphene congeners close to the primary site of contamination (OIA) and following a relatively short river borne dispersion (Ord River downstream site).

Other organochlorine pesticides

Other organochlorine pesticides in the samples, present at much lower concentrations (Table 2) than *p,p′*-DDE and toxaphene, presumably reflect a combination of global background and local usage. Some of these organochlorine pesticides were widely used in Australian agriculture and in pest control industries in the 1960s and early 1970s. This usage fell markedly during the late 1970s, and by 1981, with the exception of some tropical (including use on sugarcane) and other minor applications, most agricultural uses had been deregistered. Use to kill termites ended by 1995 with the exception of Mirex, which was employed until 1997.[37]

Blood serum analyses of the crocodiles

Results for the analyses of blood serum parameters, including testosterone and β-estradiol, are summarised in Table 5 (full details of analyses for all crocodiles are given as supplementary material†). It was apparent that the concentrations of the sex hormones varied greatly, depended on the age and breeding state of the individual animals, and that there was no discernable relationship with pesticide concentrations. The small number of animals sampled was, however, not conducive to revealing any possible relationship. Relationships between environmental contaminants and plasma sex steroid concentrations have, however, been reported for juvenile American alligators[38–40] and a correlation between the concentrations of *p,p′*-DDE and testosterone was shown for Dall's porpoises from the north Pacific Ocean.[41]

Uric acid, cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations in blood serum of freshwater crocodiles, *C. johnstoni*, all showed significant differences between pairs of locations (Student's *t* tests). Uric acid concentrations in animals from the OIA were different from those from the lower Ord (significant at 0.05% level); as

were OIA *vs.* upper Ord (0.01%). Cholesterol in OIA animals was different from lower Ord animals (0.05%), and from upper Ord animals (0.01%). HDL and LDL concentrations were both different between OIA and lower Ord crocodiles (0.05% in each case), and between OIA and upper Ord crocodiles (0.01% in each case). It is unclear, however, if any of these differences are related to pesticide concentrations. Certainly, within each location there was no relationship between the values of these parameters and the concentrations of *p*,*p*′-DDE and total toxaphene.. It has been shown that the blood chemistry of crocodiles is affected by stress[42–44] and it is possible that the results for the animals from the upper river location may reflect the prolonged stress caused by the method of sampling (noosing) and their transport to a central laboratory live in wet hessian sacks. Other animals were shot, died instantly, and samples were immediately taken.

Gonad histology

No significant differences were observed between the histology of testes and ovaries of exposed and control animals (Tables 6 and 7). No relationships with *p*,*p*′-DDE or toxaphene concentrations were observed. As for the blood serum parameters, it is possible that the small number of animals sampled and their variations in breeding state may have masked any effects. However, we can say that the very high levels of the known endocrine disrupting *p*,*p*′-DDE and toxaphene have not resulted in gross observable pathological effects on the crocodile gonads.

Summary and directions for future research

The crocodile populations in the lower Ord River in the north of Western Australia have provided an unusual, perhaps unique, opportunity to study the long term consequences, in terms of accumulation, transformation and possible effects, of known amounts of DDT and toxaphene applied over a known period some 30 or so years before sampling.

The most striking results of the study are the very high, some of the highest ever recorded in wildlife, concentrations of *p*,*p*′-DDE and some toxaphene congeners; and the lack of any obvious effects of these on the crocodiles that accumulated them. All were apparently healthy, their blood parameters were not different from those of control animals in any way that could easily be ascribed to pesticide burdens. The lack of any obvious differences between heavily contaminated animals and controls was also found for gonad histology. The sample size was small the variations in sexual maturity and breeding state of the animals effectively reduced the size of each category still further. The small sample size also made it pointless to look for the type of physical effects reported by Guillette and his co-workers.[38–40,45]

Table 6 Histological analyses of ovaries of female crocodiles *Crocodylus johnstoni* and *C. porosus*

Sample No.	Species	Location	DDE[a]	Description	Season	Ovarian follicle disappearance[b]	Vitello-genesis[b]	Corpus albicans[b]	Lymph duct dilatation[b]	Blood vessel hyalinisation[b]	Adrenal gland vacuolation[c]
1	*C. johnstoni*	Lower Ord	4+	Mature	Post breeding	+	+++	++	+	—	0
2	*C. johnstoni*	Lower Ord	4+	Adolescent	Post breeding	+	—	+++	—	—	0
3	*C. johnstoni*	Lower Ord	4+	Mature	Post breeding	+	++	++	++	++	0
4	*C. johnstoni*	Lower Ord	5+	Adolescent	Post breeding	+	+	+++	+	—	+++
11	*C. johnstoni*	OIA	3+	Adolescent	Post breeding	+	++	++	++	—	++
21	*C. johnstoni*	Upper Ord (control)	+	Juvenile	Breeding	—	—	—	++	—	+
22	*C. johnstoni*	Upper Ord (control)	—	Adolescent	Breeding	+	+	++	++	—	+
23	*C. johnstoni*	Upper Ord (control)	—	Mature	Post breeding	++	+	+	++	—	0
24	*C. johnstoni*	Upper Ord (control)	—	Mature	Post breeding	++	+++	—	++	—	+
31	*C. porosus*	Lower Ord	2+	Juvenile	Breeding	++	—	+	++	+	+++
32	*C. porosus*	Lower Ord	2+	Adolescent	Breeding	+	—	++	++	—	0
33	*C. porosus*	Lower Ord	3+	Adolescent	Post breeding	++	—	+	++	—	+++
34	*C. porosus*	Lower Ord	2+	Mature	Breeding	+	++	++	++	—	0

[a] DDE concentration in visceral fat on an extractable lipid basis. −, <10 ng g^{-1}; +, 10 to 100 ng g^{-1}; 2+, 100 to 1000 ng g^{-1}; 3+, 1000 to 10 000 ng g^{-1}; 4+, 10 000 to 100 000 ng g^{-1}; 5+, >100 000 ng g^{-1}. [b] The extent of disappearance of ovarian follicles, of vitellogenesis, of lymph duct dilatation, and the existence and number of corpora albicantia, of blood vessel hyalinisation are indicated by the symbols: −, +, ++, +++. − indicates absence; +, ++ and +++ represents increasing incidence of the histological condition. All are within the normal range. [c] The extent of vacuolation of adrenal gland is indicated by the symbols: + (normal), ++, +++. Samples lacking an adrenal gland are indicated by 0.

Table 7 Histological analyses of testes of male crocodiles *Crocodylus johnstoni* and *C. porosus*

Sample No.	Species	Location	DDE[a]	Description	Season	Seminiferous tubules[b]	Epithelium vacuolation[c]	Spermato-genesis[c]	Interstitial tissue[c]	Parasitic granulo-mata[d]	Parasites in adrenal gland[e]
5	*C. johnstoni*	Lower Ord	3+	Adolescent	Post breeding	Medium	+++	—	++	—	0
6	*C. johnstoni*	Lower Ord	5+	Mature	Post breeding	Large	+++	+	—	+	0
7	*C. johnstoni*	Lower Ord	4+	Mature	Post breeding	Large	+++	+	+	—	0
8	*C. johnstoni*	Lower Ord	4+	Mature	Post breeding	Large	+++	++	++	+	0
9	*C. johnstoni*	Lower Ord	4+	Mature	Post breeding	Medium	+++	—	++	++	+
10	*C. johnstoni*	Lower Ord	4+	Mature	Post breeding	Medium	+	—	+	+	—
12	*C. johnstoni*	OIA	3+	Adolescent	Post breeding	Small	+++	—	++	+++	+
13	*C. johnstoni*	OIA	4+	Adolescent	Post breeding	Medium	+	—	+++	+++	+
14	*C. johnstoni*	OIA	4+	Juvenile	Post breeding	Small	+	—	++	++	+++
15	*C. johnstoni*	OIA	3+	Juvenile	Post breeding	Small	+++	—	++	++	—
16	*C. johnstoni*	OIA	4+	Juvenile	Post breeding	Medium	++	—	++	+	—
17	*C. johnstoni*	OIA	3+	Adolescent	Post breeding	Medium	++	—	++	++	+
18	*C. johnstoni*	OIA	4+	Mature	Post breeding	Medium	++	—	++	++	—
19	*C. johnstoni*	OIA	4+	Adolescent	Post breeding	Medium	+	—	++	+++	—
20	*C. johnstoni*	OIA	4+	Adolescent	Post breeding	Small	+	—	+	++	++
25	*C. johnstoni*	Upper Ord (control)	—	Juvenile	Post breeding	Small	++	—	+++	+	—
26	*C. johnstoni*	Upper Ord (control)	—	Adolescent	Post breeding	Medium	++	—	++	—	—
27	*C. johnstoni*	Upper Ord (control)	—	Juvenile	Post breeding	Small	+++	—	+++	—	—
28	*C. johnstoni*	Upper Ord (control)	—	Juvenile	Post breeding	Small	++	—	++	—	—
29	*C. johnstoni*	Upper Ord (control)	No data	Juvenile	Post breeding	Small	+++	—	+	—	—
30	*C. johnstoni*	Upper Ord (control)	—	Juvenile	Post breeding	Small	+	—	+	—	+
35	*C. porosus*	Lower Ord	2+	Juvenile	Breeding	Small	+++	—	+	—	—
36	*C. porosus*	Lower Ord	3+	Adolescent	Breeding	Small	+++	—	++	—	0
37	*C. porosus*	Lower Ord	2+	Juvenile	Breeding	Small	+++	—	+	—	—
38	*C. porosus*	Lower Ord	4+	Mature	Breeding	Large	—	+++	—	—	0
39	*C. porosus*	Lower Ord	2+	Juvenile	Breeding	Small	+++	—	+	++	—
40	*C. porosus*	Lower Ord	4+	Adolescent	Post breeding	Large	—	++	—	+	0

[a] DDE concentration in visceral fat on an extractable lipid basis. −, <10 ng g^{-1}, +, 10 to 100 ng g^{-1}; 2+, 100 to 1000 ng g^{-1}; 3+, 1000 to 10 000 ng g^{-1}; 4+, 10 000 to 100 000 ng g^{-1}; 5+, >100 000 ng g^{-1}. [b] Seminiferous tubules were classified as large, medium or small. All may be regarded as normal. [c] The extent of vacuolation in the seminiferous tubular epithelium and of spermatogenesis, and the volume of interstitial tissue in the testes are indicated by the symbols: −, +, ++, +++. All are likely to be within the normal range. [d] The development of parasitic granulomata in the interstitial tissue of the testes was classified as -, +, ++, +++. [e] Parasitism in the adrenal gland was classified as −, +, ++, +++ for absence and increasing incidence of parasites. Samples lacking an adrenal gland are indicated by 0.

The population dynamics of *C. johnstoni* in the Ord River have not been studied and there is no historical record against which to measure any possible changes. This goes for sex ratios as well as animal numbers. Crocodiles lack sex chromosomes and sex is determined by nest temperature, although the mechanisms are complex and poorly understood. For both *C. johnstoni* and *C. porosus* females are produced at low and high temperatures and males at intermediate temperatures. The sex ratios of *C. porosus* in the rivers of the Kimberley of Western Australia are variable with a 2 : 1 ratio of males to females reported for the Ord River.[32] Such environmentally determined variability, which will presumably apply to

C. johnstoni as well as *C. porosus*, will confound efforts to determine the possible effects of xenoestrogens. Nevertheless, possible effects of the high concentrations of *p,p′*-DDE and toxaphene in Ord River crocodiles is most likely to be seen at a population level, and study of the population biology of, in particular, *C. johnstoni*, in the rivers of the east Kimberley of Western Australia is urgently needed.

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