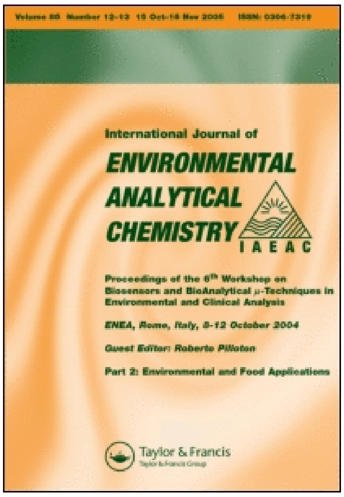

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Persistent organochlorine pesticides and their metabolites in alligator livers from Lakes Apopka and Woodruff, Florida, USA

Arthur W. Garrison[a]; Louis J. Guillette Jr[b]; Thomas E. Wiese[c]; Jimmy K. Avants[d]
[a] National Exposure Research Laboratory, US Environmental Protection Agency, Athens, GA 30605, USA [b] Department of Zoology, University of Florida, Gainesville, FL, USA [c] Xavier University of Louisiana College of Pharmacy, New Orleans, LA, USA [d] Senior Service America, US EPA, Athens, GA, USA

To cite this Article Garrison, Arthur W. , Guillette Jr, Louis J. , Wiese, Thomas E. and Avants, Jimmy K.(2010) 'Persistent organochlorine pesticides and their metabolites in alligator livers from Lakes Apopka and Woodruff, Florida, USA', International Journal of Environmental Analytical Chemistry, 90: 2, 159 — 170

To link to this Article: DOI: 10.1080/03067310902977526

URL: http://dx.doi.org/10.1080/03067310902977526

Intern. J. Environ. Anal. Chem.
Vol. 90, No. 2, 15 February 2010, 159–170

Persistent organochlorine pesticides and their metabolites in alligator livers from Lakes Apopka and Woodruff, Florida, USA

Arthur W. Garrison[a]*, Louis J. Guillette Jr[b],
Thomas E. Wiese[c] and Jimmy K. Avants[d]

[a]*National Exposure Research Laboratory, US Environmental Protection Agency, Athens, GA 30605, USA;* [b]*Department of Zoology, University of Florida, Gainesville, FL, USA;* [c]*Xavier University of Louisiana College of Pharmacy, New Orleans, LA, USA;* [d]*Senior Service America, US EPA, Athens, GA, USA*

(*Received 29 October 2008; final version received 10 April 2009*)

Reproductive disorders in American alligators (*Alligator mississippiensis*) inhabiting Lake Apopka, Florida, have been observed for several years. Such disorders are hypothesised to be caused by endocrine disrupting contaminants occurring in the lake due to pesticide spills and runoff from bordering agricultural lands. Various studies have resulted in identification of several persistent chlorinated organic pollutants, some of them known endocrine disrupters, in various alligator tissues and fluids. In this report, livers from 12 juvenile alligators inhabiting Lake Apopka and 10 from Lake Woodruff, a control lake, were extracted and analysed using gas chromatography–mass spectrometry with chiral GC columns for identification of both chiral and non-chiral organochlorine pesticides (OCPs, including their metabolites); in so doing, the enantiomer fractions of any chiral OCPs identified were also measured. In Lake Apopka, *p,p′*-DDE was the most prominent OCP identified, being found in all samples at concentrations ranging from 4 to 779 ng g^{-1}, based on wet weight of the liver samples. *Trans*- and *cis*-nonachlor were also detected in all samples at a concentration range of 0.3 to 64 ng g^{-1}; *p,p′*-DDD was also detected in all samples, but at an even lower concentration of 0.2 to 11 ng g^{-1}. Only 5 chiral OCPs were identified; their enantiomer fractions were mostly non-racemic, indicating pre-ingestion enantioselective biotransformation or enantioselective metabolism by the alligators. *p,p′*-Dichlorobenzophenone (*p,p′*-DCBP), a known metabolite of *p,p′*-dicofol, was detected in all but one sample; most concentrations were <1 ng g^{-1}. Dicofol is known to have been used and spilled near Lake Apopka, and is highly toxic to fish and aquatic invertebrates. Experiments showed that the *p,p′*-DCBP identified in these samples occurred via thermal degradation during GC analysis of *p,p′*-dicofol that was present in the liver sample extracts. Only 5 OCPs, at levels much below those in Lake Apopka, were found in control Lake Woodruff.

Keywords: alligators; alligator livers; OC pesticides; OCPs; chiral pesticides; enantiomers; *p,p′*-DDE; *p,p′*-DDD; *trans*-nonachlor; *cis*-nonachlor; *p,p′*-dicofol; *p,p′*-dichlorobenzophenone

---

*Corresponding author. Email: garrison.wayne@epa.gov

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310902977526
http://www.informaworld.com

1. Introduction

Reproductive disorders in American alligators (*Alligator mississippiensis*) inhabiting Lake Apopka, Florida (Figure 1), have been observed for several years [1,2]. Such disorders include decreased clutch viability, increased neonatal mortality, alterations in plasma hormone concentrations and morphological abnormalities. For example, after a dicofol spill near Lake Apopka in 1980, the number of eggs hatched *versus* laid averaged less than 20% (1983–1991), while those in Lake Woodruff, a reference site, averaged

LAKE	LATITUDE	LONGITUDE
Apopka	28¡37′N	81¡37W
Griffin	28¡50′N	81¡51W
Monroe	28¡50′N	81¡16W
Jessup	28¡44′N	81¡12W
Okeechobee	26¡56′N	80¡49′W
Orange	29¡28′N	82¡11′W
Woodruff NWR	29¡06′N	81¡25′W

Figure 1. Location of Lakes Apopka and Woodruff in Florida.

88% (1982–1990). Observed morphological abnormalities in Lake Apopka alligators include poorly organised testes and reduced phallus size of juveniles, as well as multiocytic ovarian follicles. These disorders are hypothesised to be caused by endocrine disrupting (ED) contaminants occurring in the lake due to pesticide spills and runoff from bordering agricultural lands [3], and various studies have resulted in identification of several organochlorine pollutants, some of them known ED compounds, in alligator serum [3] (Figure 2), eggs [4], and other tissue and fluid samples [5]. Most of these pollutants were organochlorine pesticides and their metabolites or transformation products, or polychlorinated biphenyls (PCBs).

Previous studies have not measured organochlorine pollutants in alligator *livers*; such data could provide important confirmation relative to proposed hepatic toxicological mechanisms. In fact, it has been shown that juvenile alligators living in various organochlorine pollutant-contaminated lakes in Florida exhibit altered patterns of hepatic testosterone biotransformation [6].

A large body of literature describes analyses and occurrences of the enantiomers of chiral organochlorine pesticides in various biota [7]. However, previous studies have not reported enantiomer composition of any chiral pesticides (or other chiral pollutants) in alligators, even though it is known that enantiomers of chiral pesticides often differ in their biological activity [8], including endocrine disruption. For example, the R-(−)-enantiomer of *o,p′*-DDT [9,10], and the (−)-enantiomer of dicofol [11] (Figure 3) have greater estrogen activity than their (+)-enantiomers. In this research, livers from 12 juvenile alligators inhabiting Lake Apopka were extracted and analysed using gas chromatography–mass spectrometry with a chiral column for separation and identification of both chiral and non-chiral organochlorine pesticides/metabolites (OCPs) and the enantiomers of any chiral OCPs. In so doing, this allowed calculation of the enantiomer fractions (EF) of any chiral pesticides identified.

2. Experimental

2.1 *Reagents, chemicals and standards*

Mussel tissue samples (SRM 1974a), certified as to concentration of a variety of OCPs, were obtained as frozen homogenates from the US National Institute of Standards and

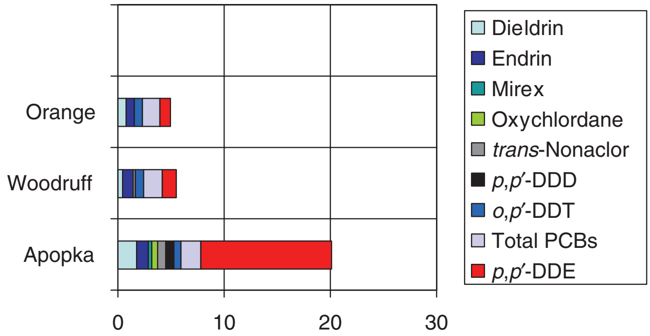

Figure 2. Mean serum contaminant concentrations (ng mL^{-1}) of OCPs (and total PCBs) in juvenile alligators from Florida lakes, 1995. From [3].

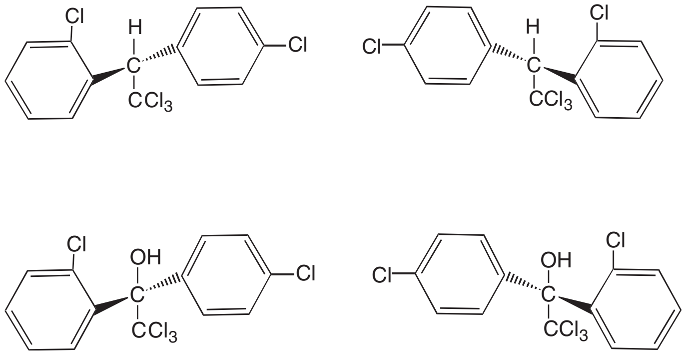

Figure 3. Enantiomers of *o,p*′-DDT (top) and *o,p*′-Dicofol (bottom).

Technology (NIST, Gaithersburg, MD, USA). Pesticide and metabolite standards were obtained from the US Environmental Protection Agency National Pesticide Standard Repository (Ft. Meade, MD, USA), from Chem Service (West Chester, PA, USA), or from Sigma Chemical Co. (St. Louis, MO, USA). Solvents and sulphuric acid were obtained from Fisher Chemicals (Fair Lawn, NJ, USA).

2.2 *Sample preparation*

Juvenile alligators (46–58 cm snout length and approximately 5–7 years of age) [12], were hand caught from Lake Apopka (28°37′N; 81°37′W), FL, USA and Lake Woodruff National Wildlife Refuge (29°06′N; 81°25′W) on the evenings of 11 September and 10 September 2001, respectively, from an airboat and transferred to the University of Florida. Within 4 hours, the animals were killed with an overdose of nembutol and necropsied under sterile conditions. Liver tissue was excised, flash frozen in liquid nitrogen, and shipped via express mail to the US EPA in Athens, GA. For analysis, the livers were freeze dried, ground with a Wiley mill, and extracted 3 times with 100 mL of hexane by sonication. The 3 extracts were combined and centrifuged, and the liquid phase was evaporated to 5 mL. Each of these combined extracts was shaken with 10 mL of 18 M H_2SO_4 to clean the sample matrix by oxidation, leaving most OCPs unaffected. The hexane layer was washed 4 times with water, dried by contact with Na_2SO_4, then evaporated to 50 µL for GC-MS analysis.

2.3 *Chromatographic instrumentation and conditions*

Chromatographic analysis of the extracts was by GC-MS with electron ionisation in the SIM mode using a BGB-172 (BGB Analytik AG, Switzerland) chiral column, which is known to separate the enantiomers of many chiral OCPs. The GC-MS instrument was a Hewlett-Packard 5973 mass spectrometer linked to a 6890 gas chromatograph equipped with the BGB 172 column. Conditions were: injection, splitless at temp. 275°C; column temp. programme, 180–220°C at 1.5° min^{-1}, followed by temp. hold for 20 min (this programme was different for chlordane – see Section 2.4); helium gas flow, 1.5 $\mathrm{mL\,min}^{-1}$; MS inlet temp., 275°; MS source temp., 230°; and fragmentation voltage, 70 eV.

Table 1. Recoveries of pesticides and metabolites from spiked Lake Woodruff alligator livers and SIM ions used in GC-MS analysis.

Pesticide	% Recovery[a]	SIM ions (GC/MS)
o,p′-DDE	58	246, 318
p,p′-DDE	52	"
o,p′-DDD	49	235, 237
p,p′-DDD	52	"
o,p′-DDT	53	"
p,p′-DDT	74	"
trans-Nonachlor	51	375, 389, 409
cis-Nonachlor	51	"
Oxychlordane	35	387, 389
trans-Chlordane	63	373, 375
cis-Chlordane	63	"
Endrin	41	263, 265

Note: [a]Recoveries for DDT derivatives are the average for 2 spiked samples.

Sample injection volume was 1 uL. Detection was by selected ion monitoring (SIM); SIM ions used for each analyte are given in Table 1. Quantification was by comparison of sample peak areas to a mixture of standards of similar concentration analysed the same day; quality control included analysis of at least one standard mixture per day as well as analysis of a standard mixture before and after each 10 samples. Standards in the mixture were referenced to a standard curve of peak area *versus* concentration for each individual analyte.

Some extracts were analysed for dicofol by HPLC using a Chiralcel®OJ chiral column (Chiral Technologies, Inc, West Chester, PA, USA), 93 : 7/hexane : ethanol eluant, and 0.8 mL min^{-1} flow rate.

2.4 *Enantiomer separation and enantiomer fractions (EF)*

Five chiral OCPs were identified in various liver samples. Enantiomers of all these were separated on the BGB-172 column. The quality of separation and enantiomer elution order, determined by analysis of separate enantiomers, are shown in Table 2. Under the typical GC conditions (Section 2.3) *o,p′*-DDT and oxychlordane enantiomers separated just to baseline. *o,p′*-DDD enantiomers separated to about 35% from peak apex, sufficient for accurate integration. Runs to include chlordane required a slow GC column temp. programme of 150 to 220°C at 0.3° min; under these conditions, the 4-enantiomer group of chlordane eluted between 148 and 153 min. Although the 4 enantiomers were separated by more than 1 min from each other the 2 *cis*-chlordane enantiomers eluted between the 2 enantiomers of *trans*-chlordane. However, each enantiomer was separated from the others almost to baseline. (In later unrelated work, using a different BGB-172 column, we were able to separate all 4 chlordane enantiomers to baseline during a time of only 68.50 to 69.95 minutes at a column temp. of 150–220° followed by a hold of 20 min with a gas flow of 1° min^{-1}).

Table 2. Quality of enantiomer separation and enantiomer elution order of chiral pesticides identified in Lake Apopka.

Pesticide	Enantiomer Separation	Elution Order
o,p′-DDT	baseline	−, +
o,p′-DDD	~35%	+, −
cis-Chlordane	~2 min	+, −
trans-Chlordane	~5 min	+, −
Oxychlordane	baseline	+, − [a]

Note: [a]From: Wiberg *et al.*, Chemosphere **45**: 843–848, 2001, using a BGB-172 column.

Enantiomer fractions were calculated as:

$$EF = [E_1]/([E_1] + [E_2])$$

where $[E_1]$ and $[E_2]$ are the concentrations of the first and second eluting enantiomers on a given chiral column.

2.5 *Quality control*

2.5.1 *Reference samples*

Seven alligator liver samples from relatively clean Lake Woodruff were analysed by the same methods used for Lake Apopka samples.

2.5.2 *Recoveries from spiked Lake Woodruff alligator livers*

After the freeze drying and grinding steps, portions of liver samples from the relatively clean Lake Woodruff alligators were spiked by syringe using 200 uL volumes of the mixed OCPs in MeOH at 2 ug mL^{-1} concentrations. Recoveries are presented in Table 1, along with the SIM ions used for GC-MS analysis.

2.5.3 *Recoveries from a surrogate*

Two NIST-certified mussel samples, surrogates for alligator livers, were analysed using the same methods as for the alligator livers, starting with the hexane extraction/sonication step. Average recoveries from the two samples, based on the NIST certified concentrations, are given in Table 3.

3. Results

3.1 *Analytical quality control*

Table 4 summarises the number of detections, with concentrations and ranges, of OCPs found in the 12 alligator livers from Lake Apopka, as well as those from the reference lake, Lake Woodruff (Figure 1); concentrations are given in ng g^{-1}, based on the wet weight of the liver samples. The much lower occurrences and concentrations of the OCPs detected in

Table 3. Recoveries of pesticides from the NIST-certified mussel samples.

Certified Pesticide	% Recovery, average
p,p′-DDT	36
p,p′-DDD	49
trans-Nonachlor	45
cis-Nonachlor	56
trans-Chlordane	63
cis-Chlordane	80

Table 4. Concentrations of OCPs in alligator livers from Florida lakes, ng g^{-1}.

	Lake Apopka ($n=12$)			Lake Woodruff ($n=8$)	
Pesticide	Detections	Average Conc.	Conc. Range	Detections	Conc.
o,p′-DDE	1	1.9	–	ND	
p,p′-DDE	12	114	4.3–778	7	0.9–4.6[a]
o,p′-DDD[b]	2	1.6	1.2–2.0	ND	
p,p′-DDD	12	2	0.2–10.7	1	0.5
o,p′-DDT[b]	1	2.3	–	ND	
p,p′-DDT	2	11.3	1.6–21.0	ND	
trans-Nonachlor	12	6.9	0.3–64.0	1	0.5
cis-Nonachlor	11	3.2	0.3–26.0	1	0.3
Oxychlordane[b]	3	10	2.5–22.0	ND	
trans-Chlordane[b]	2	16	3.4–28.6	ND	
cis-Chlordane[b]	1	1.7	–	ND	
Endrin	5	8.6	4.2–23.4	2	0.9, 27.5
p,p′-DCBP[c]	11	0.7	0.2–36	ND	

Notes: [a]Concentration range.
[b]Chiral pesticides.
[c]*p,p′*-Dichlorobenzophenone.

Lake Woodruff alligator livers relative to those in Lake Apopka livers were as expected since Lake Woodruff, located in the Lake Woodruff National Wildlife Refuge, is a much cleaner lake. This comparison thus provided some assurance of analytical quality. As shown in Table 1, recoveries of OCPs from spiked Lake Woodruff alligator livers ranged from 35% to 74%, with an average recovery of 54%, The Lake Apopka and Lake Woodruff samples were not corrected for these recoveries, so actual liver concentrations may be about twice as high for most detected compounds. It was assumed that mussel tissues with NIST certified concentrations of endogenous OCPs would serve as good surrogates for alligator livers, and thus as good QA samples. There were only 6 OCPs certified in the NIST reference samples that also occurred in the alligator livers. As seen in Table 3, average recoveries of these 6 OCPs from 2 samples of this tissue ranged from 36% to 80%, for an overall average of 55%, similar to recoveries for the same spiked compounds in the Lake Woodruff livers.

In addition to these recovery data, the chiral compounds identified in these samples – *o,p′*-DDT, *o,p′*-DDD, *cis*-chlordane, *trans*-chlordane and oxychlordane – were qualitatively confirmed by spiking at least one of the samples containing them and observing the peak height increase. Finally, *p,p′*-DDE, *p,p′*-DDD, *cis*-nonachlor and *trans*-nonachlor were qualitatively confirmed by analysis of some of the extracts by GC-ECD and matching retention times of tentatively identified pesticides with standards.

3.2 *Occurrence, concentration and EF data*

In Lake Apopka, the *p,p′*-isomers of the DDT-related compounds were found more frequently and in higher concentrations than their *o,p′*-analogs (Table 4). This is as expected, since *p,p′*-DDT comprises about 75% or more of technical grade DDT, while the remainder varies between 15 and 21% of the *o,p′*-isomer, plus minor impurities [13,14]. While the parent pesticide, *p,p′*-DDT, was found in only 2 liver samples, *p,p′*-DDE, the principal metabolite of *p,p′*-DDT in mammals [13] and fish [15], was found in all samples and in much higher concentrations, an average of $114\,\mathrm{ng\,g^{-1}}$, than any of the other OCPs, reaching a concentration of $779\,\mathrm{ng\,g^{-1}}$ in one sample. Fish rapidly accumulate DDT from aqueous media, and its major biotransformation product is DDE [15]. As well as being a metabolite of *p,p′*-DDT, *p,p′*-DDE is also found in soils and sediment after DDT weathering, so some of the liver DDE could have resulted from ingestion of sediment or biota from lower trophic levels by the alligators. Only one sample contained *o,p′*-DDE, and it was at a very low concentration. *p,p′*-DDD, the principal product of DDT in reducing environments [16], was found in all samples, but its highest concentration was only about $11\,\mathrm{ng\,g^{-1}}$. This compound could also have been directly ingested through sediment. It also could have been an alligator metabolite, but this is less likely since DDD is known to be metabolised in mammals to an easily eliminated secondary metabolite and is rarely found as a stored metabolite [17]. This was also shown to be true in fish, where DDD is a major metabolite, but has a significantly higher elimination rate than DDE [15].

o,p′-DDT and -DDD, the chiral members of the DDT group, were found in only 1 and 2 samples, respectively. The enantiomer fraction (EF) of the *o,p′*-DDT is 0.47, the same as the standard, 0.471 (Table 5), meaning that the sample has not been depleted in either enantiomer, although non-selective biotransformation could have occurred. The two EF values of *o,p′*-DDD were different; one was 0.49, similar to the standard of 0.481, while the other was considerably higher, 0.51, indicating stereoselective depletion of the enantiomer.

Table 5. Enantiomer fractions of chiral OCPs in standards and alligator livers from Lake Apopka.

Pesticide	Standard EF	EF in Livers
o,p′-DDD	0.481 ± 0.004 (6)[a]	0.51[b], 0.49[b]
o,p′-DDT	0.471 ± 0.005 (6)	0.47
cis-Chlordane	0.499 ± 0.007 (4)	0.47
trans-Chlordane	0.479 ± 0.012 (4)	0.50[b], 0.50[b]
Oxychlordane	0.492 ± 0.003 (6)	0.532 ± 0.001 (3)[a], 0.52[b], 0.47[b], 0.59[b]

Notes: [a](*n*).
[b]These EF values are from separate liver samples.

Table 4 shows that *p,p′*-DDE is also the most abundant DDT analog in Lake Woodruff, as is was in Lake Apopka, and was found in all seven liver samples. This is as expected since any level of DDT contamination would lead to eventual bioaccumulation of more of the DDE metabolite than the parent DDT. However, the concentration in much cleaner Lake Woodruff was very low relative to that in Lake Apopka. There was no DDT found in Lake Woodruff alligator livers, but there was a small amount of *p,p′*-DDD in one liver sample.

Cis- and *trans*-nonachlor are closely related to chlordane and are components of commercial chlordane formulations [18], occurring therein to about 5% to 7%. Both were detected in all but one liver sample and, although their average concentrations were low, they ranged to a higher concentration than most of the other OCPs found (Table 4). *Trans*-nonachlor, an endocrine disrupter [18] that exhibits strong binding to estrogen receptors [19], was 2–3 times more concentrated than *cis*-nonachlor, reaching a concentration of 64 ng g^{-1}. There were only 3 occurrences of chlordane (one *cis* and two *trans*). It appears that most chlordane that may have occurred in the alligator's environment had been degraded or transformed to other compounds, leaving residues of the more persistent nonachlor for alligator uptake; on the other hand, any chlordane that was ingested by the alligators could have been transformed or eliminated by the animals. *Cis*- and *trans*-chlordane are chiral pesticides; the *cis*-isomer occurred once with an EF of 0.47 (Table 5), indicating enantioselective biotransformation relative to its standard of 0.499. *Trans*-chlordane occurred twice, with the same EF value of 0.50, relative to the standard value of 0.479. Apparently both compounds were biotransformed enantioselectively. Such transformation of the chlordanes could have occurred in the lake environment before ingestion or in the mammalian system. Oxychlordane, a chiral molecule, is the only chlordane metabolite detected. It was detected in 4 samples, and in all cases occurred with a non-racemic EF relative to the standard value of 0.492 (Table 5); this indicated either formation from chlordane via an enantioselective mechanism, or enantioselective biotransformation of itself.

Small concentrations of *cis*- and *trans*-nonachlor were also found in one Lake Woodruff liver sample (Table 4), implying previous contamination by chlordane formulations. Endrin, a non-chiral OCP, was found in 5 Lake Apopka samples at a concentration range of 4–23 ng g^{-1}. It was found in 2 Lake Woodruff samples; the concentration in one sample was very low, but the other sample had an even higher concentration than any of the Lake Apopka samples, 28 ng g^{-1}.

3.3 *Dicofol and dichlorobenzophenone*

Dicofol, an important currently used fungicide, was formerly synthesised from technical grade DDT, and from 5 to 20% of DDT remained in the technical dicofol. Modern processes, however, can produce technical grade dicofol that contains less than 0.1% of DDT. On the other hand, dicofol use has been shown to be a significant contributor to current DDT pollution in China [20]. Technical DDT contains, in addition to the active *p,p′*-isomer, from 15% to 30% of the *o,p′*-isomer [13,20]. Because during synthesis an average of 93% of *p,p′*-DDT is converted to *p,p′*-dicofol while only 37% of *o,p′*-DDT is converted to *o,p′*-dicofol, a smaller percentage of *o,p′*-dicofol occurs in dicofol than the percentage of *o,p′*-DDT in technical DDT [20]. However, another source [21] indicates that technical dicofol contains 20% of the *o,p′*-isomer. In any case, *o,p′*-dicofol,

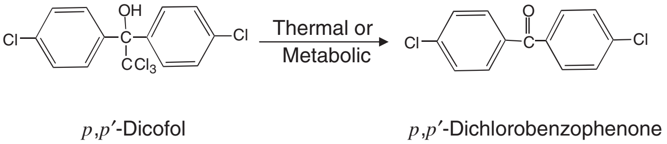

Figure 4. Conversion of *p,p*′-dicofol to *p,p*′-dichlorobenzophenone.

a suspected endocrine disruptor, is a chiral molecule (Figure 3). The (−)-enantiomer has been shown to have more estrogen activity toward the human estrogen receptor *in vitro* than does the (+)-enantiomer [11]. However, *o,p*′-dicofol was not detected in the alligator livers by our methodology.

On the other hand, we believe that *p,p*′-dicofol was in the liver samples. Dicofol itself cannot be analysed by GC; it thermally degrades to dichlorobenzophenone (DCBP) (Figure 4) [20], which can be analysed as a dicofol surrogate [21]. Coincidentally, this product is also the main metabolite of dicofol in many plants and animals [22]. We did not detect either *o,p*′- or *p,p*′-dicofol by our GC-MS analytical method, but did detect small amounts of *p,p*′-DCBP in 11 of the 12 alligator livers (Table 4); no *o,p*′-DCBP was detected. However, based on the thermal and metabolic properties of dicofol, the origin of *p,p*′-DCBP was problematic. In addition, our sample cleanup procedure included washing with conc. sulphuric acid, which could conceivably have also degraded any dicofol in the sample. But processing a dicofol standard through the entire analytical protocol, followed by HPLC analysis for dicofol, resulted in only 12% loss of dicofol with no appearance of *p,p*′-DCBP. However, the acid wash did completely degrade a standard of *p,p*′-DCBP. It appears, therefore, that since (1) most of the dicofol persists through our sample preparation procedure, and (2) the DCBP is destroyed by the acid wash step of the procedure, then *p,p*′-dicofol must have been present in the alligator livers instead of *p,p*′-DCBP, persisted through the sample preparation procedure, and been observed as *p,p*′-DCBP after thermal degradation during GC injection. Any o-p′- or *p,p*′-dicofol in the livers was apparently below our HPLC detection limits (about $0.1\,\mu g\,g^{-1}$); otherwise these compounds, instead of the DCBP, would have been detected directly by HPLC. Finally, no dicofol or DCBP were found in the Lake Woodruff reference liver samples.

The occurrence of *p,p*′-dicofol in the alligator livers is not unexpected since there was an extensive spill of that pesticide into Lake Apopka in 1980 [1], before the use of modern dicofol manufacturing processes. The demonstrated binding affinity of dicofol to estrogen receptors [11,19], leads to speculation as to its role in the abnormal reproductive development of alligators in this lake [1,2].

3.4 *Comparison with 1995 serum samples*

To the best of our knowledge, there are no other data in the literature on Lake Apopka alligator liver concentrations of OCPs. We can, however, compare the concentrations measured in these fall 2001 liver samples (Table 4) with those of serum samples from alligators collected in September 1995 (Figure 2) [3]. The liver samples contained higher levels of all the measured OCPs. For example, for liver *versus* serum: *p,p*′-DDE, 114 *versus* ~13; *trans*-nonachlor, 6.9 *versus* ~1; endrin, 8.6 *versus* ~2; and *p,p*′-DDD, 2 *versus* ~1 $ng\,g^{-1}$. DCBP was found in the liver samples, but no dicofol or DCBP were found in the serum samples. At a given time, liver concentrations would be expected to be higher than those of serum and, in this case, with 6 years' difference in sample collection and assuming no further OCP contamination of Lake Apopka, it is not clear whether these data imply reduction in OCP burden in the alligators over this time frame.

4. Conclusions

- OCPs, including a few chiral ones, are in the liver tissue of alligators in Lake Apopka. They appear to be associated with historical local pesticide use and pesticide spills since very low levels were found in alligator livers from relatively uncontaminated Lake Woodruff.
- EF values of the 5 different chiral OCPs detected indicated enantioselective microbial or mammalian biotransformation for most of them, particularly oxychlordane.
- Dicofol, a known endocrine disruptor, also occurs in the alligator livers, but is thermally degraded to dichlorobenzophenone in the GC injection port. Dicofol must be analysed by HPLC, preferably by HPLC-MS-MS. In some research in which GC is used for analysis, dichlorbenzophenone may have been observed, but was incorrectly reported as dicofol.
- More comprehensive analysis of the exposure, metabolism and multiple molecular mechanisms of toxicity of chiral OCPs are needed, especially in diverse wildlife species that serve as effective environmental sentinels.

Acknowledgements and disclaimer

This manuscript has been reviewed in accordance with the US Environmental Protection Agency's peer and administrative review policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. We appreciate the advice of Sam Dutton of the US EPA Region IV SESD laboratory on sample cleanup using sulphuric acid.

References

[1] L. Guillette Jr, D. Crain, M. Gunderson, S. Kools, M. Milnes, E. Orlando, A. Rooney, and A. Woodward, Amer. Zool. **40**, 438 (2000).
[2] L. Guillette Jr, T. Gross, G. Masson, J. Matter, H. Percival, and A. Woodward, Environ. Health Perspec. **102**, 680 (1994).
[3] L. Guillette Jr, J. Brock, A. Rooney, and A. Woodward, Arch. Environ. Contam. Toxicol. **36**, 447 (1999).
[4] R. Rauschenberger, J. Wiebe, M. Sepulveda, J. Scarborough, and T. Gross, Environ. Sci. Technol. **41**, 5559 (2007).
[5] USGS, Biological and ecotoxicological characteristics of terrestrial vertebrate species residing in estuaries: American Alligator, http://www.pwrc.usgu.gov/bioeco/amallig.htm
[6] M. Gunderson, G. LeBlanc, and L. Gillette Jr, Env. Health Perspec. **109**, 1257 (2001).
[7] C. Wong, F. Lau, M. Clark, S. Mabury, and D. Muir, Environ. Sci. Technol. **36**, 1257 (2002).
[8] A. Garrison, Environ. Sci. Technol. **40**, 16 (2006).

[9] T. Wiese and S. Nehls, Enantiomer selective estrogen and antiandrogen activity of chiral pesticides. Symposia on Chiral Pollutants: Enantioselectively and its Consequences. SETAC National Meeting, 10–15 Nov. 2001, Baltimore, MD.

[10] W. McBlain, Life Sci. **40**, 215 (1987).

[11] P. Hoekstra, B. Burnison, A. Garrison, T. Neheli, and D. Muir, Chemosphere **64**, 174 (2006).

[12] M. Milnes, A. Woodward, A. Rooney, and L. Guillette, Comp. Biochem. Physiol. A **131**, 923 (2002).

[13] T. Roberts and D. Hutson, editors, *Metabolic Pathways of Agrochemicals*, *Part 2*, *Insecticides and Fungicides* (Royal Society of Chemistry, Cambridge, UK, 1999), pp. 150–158.

[14] A. Garrison, V. Nzengung, J. Avants, J. Ellington, W. Jones, D. Rennels, and N. Wolfe, Environ. Sci. Technol. **34**, 1663 (2000).

[15] R. Kwong, P. Yu, P. Lam, and W. Wang, Environ. Tox. Chem. **27**, 2053 (2008).

[16] G. You, G. Sayles, M. Kupferle, I. Kim, and P. Bishop, Chemosphere **32**, 2269 (1996).

[17] D. Morgan and C. Roan, Nature **238**, 221 (1972).

[18] G. Bondy, I. Curran, J. Doucet, C. Armstrong, L. Coady, L. Hierlihy, S. Fernie, P. Robertson, and M. Barker, Food Chem. Toxicol. **42**, 1015 (2004).

[19] P. Vonier, Food Chem. Toxicol. **35**, 430 (1997).

[20] X. Qiu, T. Zhu, B. Yao, J. Hu, and S. Hu, Environ. Sci. Technol. **39**, 4385 (2005).

[21] K. MacLellan, D. Bird, D. Fry, and J. Cowles, Arch. Environ. Contam. Toxicol. **30**, 364 (1996).

[22] T. Roberts and D. Hutson, editors, *Metabolic Pathways of Agrochemicals*, *Part 2*, *Insecticides and Fungicides* (Royal Society of Chemistry, Cambridge, UK, 1999), pp. 159–160.