Relationships among organochlorines and lipid classes in two demersal fish species from southern California

Ami K Groce[1], M. James Allen and Alexander Bolkhovitinov[2]

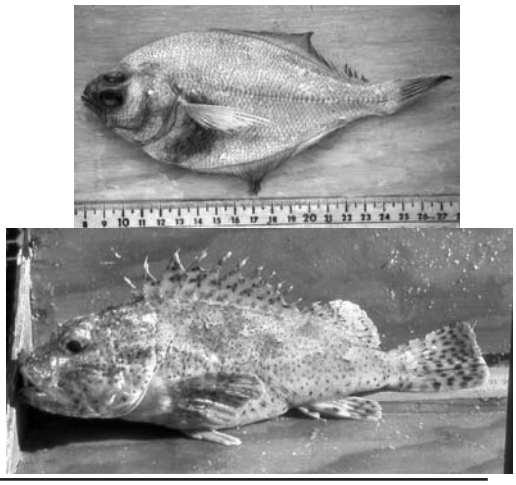
ABSTRACT - Although the potential influence of lipid reservoirs on bioaccumulation in fish is known, there is little information on how differences in lipid classes, such as triacylglycerol, may affect bioaccumulation. The objective of this study was to investigate the potential relationships between the organochlorines 1,1,1-trichloro-2,2-bis(pchlorophenyl)ethane (DDT) and polychlorinated biphenyls (PCB) and the amount and composition of lipids present in two species of fish. Organo-chlorines, total lipid, and major lipid classes were quantified from liver and muscle samples from California scorpionfish (*Scorpaena guttata*) and longfin sanddab (*Citharichthys xanthostigma*) collected near San Diego, California, in July 2000. Detection rates and concentrations of DDT and PCB were higher in liver tissues than in muscle tissues, and corresponded to higher concentrations of total lipid and triacylglycerol. However, the concentrations of the organochlorines were not correlated with the amount of lipid present.

INTRODUCTION

Contaminant concentrations in a fish can depend on several factors in addition to concentrations in the environment. Other factors include the mode and intensity of exposure, the chemical characteristics of the contaminant, and the ability of a particular species to metabolize and excrete the contaminant (Mann and Ajani 1991). The mode and intensity of exposure for a species depends on its habitat, movement, age, and choice of food (Otway 1991). The competing rates of uptake and elimination are determined by physiological and biochemical processes, which are species and age dependent (Barron 1990).

The lipid content of a fish is an important factor because organochlorine contaminants like PCBs and DDT are hydrophobic and demonstrate high affinity for lipids (Swackhammer and Hites 1988, Barron 1990, Gutenmann *et al.* 1992, Pastor *et al.* 1996). The most important uptake mechanism for organochlorines is that they partition into lipids, because this relationship was the driving influence behind other factors thought to impact the bioaccumulation of organochlorines (Phillips 1995). Further, differences in the lipid content of tissues have been identified as the primary reason for differential organochlorine accumulation (Phillips 1991, 1995).

Some studies have demonstrated that contaminant variability among samples is not always explained by differences in lipid content (Nowak 1991, COSD 1998). Van Wezel and Opperhuizen (1995) demonstrated with their work on lipid/water partition coefficients that lipids cannot be considered as a uniform compartment in fishes due to the distinct properties



[1]*City of San Diego, Metropolitan Wastewater Department, Environmental and Technical Services Division, San Diego, CA*
[2]*Amvac Chemical Company, Los Angeles, CA*

of different lipid classes (Lovern 1964, Henderson and Tocher 1987). Ewald and Larsson (1994) found that PCBs partitioned higher to neutral lipid than to phospholipid, and that lipid/water partitioning coefficients were higher with longer lipid-carbon chains. Since total lipid is comprised of several classes, some with greater affinity for hydrophobic compounds, it is possible that contaminant variability may be more accurately described by lipid class (e.g., triacylglycerol, a common hydrophobic storage lipid) rather than total lipid content. The objectives of this study were to test the hypothesis that contaminant bioaccumulation within and between two demersal fish species varied due to differences in triacylglycerol concentrations.

METHODS

Ten California scorpionfish (*Scorpaena guttata*) and 10 longfin sanddabs (*Citharichthys xanthostigma*) were collected by otter trawl in July 2000 at 10 sites located on the coastal shelf off San Diego. This area is known to have consistently low levels of DDT and PCB sediment contamination (COSD 2001). The lengths of specimens included in this study were limited as much as possible to minimize differences in size and age. Scorpionfish were between 17 and 22 cm (standard length size class) and 2 to 4 years (ages estimated from length; Love *et al*. 1987) and longfin sanddabs were between 11 and 15 cm (standard length size class) and 2 and 5 years (ages estimated from length; Groce 2002). At sea, fish were sealed in plastic Ziploc® bags and placed on dry ice for transport to the lab. To prevent lipid degradation, the fish were then placed immediately in the laboratory freezer and stored frozen at -80°C until processed.

The standard length (mm SL) and weight (g) of individual fish were recorded prior to processing. Whole livers and a section of muscle tissue were removed from each fish and weighed. A small amount of liver and muscle tissue was then removed from each fish, weighed, and placed into solvents set up for lipid extraction. The remaining liver and muscle tissues were placed in acetone-rinsed glass jars and stored at -20°C until used for organochlorine quantification (not more than six months).

All liver and muscle tissue samples were extracted quantitatively using a modified Bligh and Dyer (1959) one-phase methanol:chloroform:water extraction (2:1:0.8 v/v/v); samples were extracted overnight and phases separated the following day by the addition of chloroform and water (final solvent ratio, 1:1:0.9 v/v/v methanol:chloroform:water). All solvents used were spectrophotometric grade. The total solvent extract (TSE) was concentrated (solvents removed *in vacuo*) at 30°C under nitrogen. Total lipid was determined gravimetrically as percent wet weight.

An aliquot of the TSE (in chloroform) was analyzed using an Iatroscan MKV TH10 TLC-FID analyzer to quantify individual lipid classes (Volkman and Nichols 1991). Subsamples of each composite were applied in duplicate to silica gel SIII chromarods (5 µm particle size) using 1-µl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for lipid separation was hexane/diethyl ether/acetic acid (60:17:0.1 v/v/v), a mobile phase resolving nonpolar compounds such as wax esters (WE), sterol esters (SE), and triacylglycerol (TAG), free fatty acids (FFA), and sterols (ST). The chromarods were oven dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The flame ionization detector (FID) was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, and triolein; 0.1-12 µg range). Peaks were quantified on an IBM-compatible computer using TSCAN3 software. Iatroscan results are generally reproducible to ± 5 to 10% (Volkman and Nichols 1991, Nichols *et al.* 1998).

Liver and muscle samples were thawed and weighed. Each sample was ground with potassium oxalate and Celite 545 to smooth powder and transferred to a syringe with a modified and preconditioned C18 SPE column attached to it. The C18 column was modified by adding Celite 545 before and after the C18 silica layer. Celite and C18 were incorporated to trap lipid in the samples. Organochlorine pesticides were eluted with 20% water in acetone. The eluate was partitioned in a separatory funnel with acetone/methylene chloride (0.5:1 v/v). The organic layer was collected in a flask and then the aqueous layer was partitioned a second time with acetone/methylene chloride (1:1 v/v). The bottom layer was combined with the first extract. The final extract was turbo-evaporated at 35°C and solvent exchanged to 1 mL in hexane.

Gas chromatographic (GC) analyses were performed in a Varian 3800 GC equipped with a DB XLB capillary column (60 m x 0.32 mm x 025 µm), a Varian Saturn 2000 Ion Trap, and a Varian 1079

SPI injector. Helium was used as a carrier gas at constant flow of 1.3 mL/min. Samples were fortified with PCB30 and PCB205 as internal standards and injected in split mode (programmed as follows: 1:4 for 0.3 min; off for 2 min; 1:20 for 20 min) at an injector with the next temperature profile: 60°C for 0.3 min; 60-310°C at 200°C/min. Oven temperature profile was 60°C for 1 min; 60-180°C at 15°C/min; 180-280°C at 2°C/min; 280-310°C at 5°C/min; 310°C for 3 min. Chromatograms were processed with Varian Saturn 2000 Workstation Software. Blank, duplicate, and spike samples were prepared and analyzed with each batch of eight samples. Six DDT isomers and derivatives and 27 PCB congeners were quantified (Table 1).

Linear regressions were used to test the hypothesis that DDT and PCB concentrations varied with the amount of total lipid (TL) or TAG present in muscle and liver tissues from California scorpionfish and longfin sanddab. Dependent variables included total DDT (tDDT, which equaled p,p-DDE for all samples) and total PCB (tPCB, the sum of all PCB congeners detected in each sample) (Table 1). The five most abundant PCB congeners were also used individually as dependent variables. Only total lipid was used as the independent factor in models of muscle data for both species since very little TAG was present.

Table 1. Organochlorines quantified in study of relationships of organochlorines and lipid classes in two demersal fish species from southern California.

Total DDT	PCB Congeners*			
o,p' - DDE	PCB8	PCB66	PCB126	PCB187
o,p' - DDD	PCB18	PCB77	PCB128	PCB188
o,p' - DDT	PCB28	PCB87	PCB138	PCB195
p,p' - DDE	PCB29	PCB101	PCB153	PCB200
p,p' - DDD	PCB44	PCB104	PCB154	PCB206
p,p' - DDT	PCB50	PCB105	PCB170	PCB209
	PCB52	PCB118	PCB180	

* summed for total PCB

Results

DDT and PCB congeners were detected in 100% of the California scorpionfish and longfin sanddab liver samples analyzed in this study (Table 2). PCB118, PCB138, PCB180, and PCB187 were also detected frequently (>50% of the samples). Total lipid concentrations in liver were highly variable, and consisted almost completely of TAG.

DDT was detected in 90% of the scorpionfish and 100% of the longfin muscle samples, but at substantially lower concentrations than those found in livers. PCBs were not detected in any of the muscle samples. Muscle tissue lipids also were found in low concentrations relative to the livers, and were composed primarily of phospholipids (PL).

For the fishes sampled in this study, the concentrations of total DDT, total PCB, and the four most abundant PCB congeners did not correlate with the amount of TAG present in the liver tissues (Table 3). The relationship between tDDT or tPCB and TL was not substantially different than between tDDT or tPCB and TAG, because the amount of TL was strongly correlated to the amount of TAG present in the liver (Groce 2002). In addition, there was no relationship between muscle DDT and total lipid.

Discussion

No relationship was found between concentrations of DDT and PCB and the amount of TL or the amount of TAG present in liver or muscle tissues from either California scorpionfish or longfin sanddab. If TAG (or total lipid) was the driving factor behind organochlorine variability, a strong linear relationship would be expected between the two.

A possible contributing factor may have been that these organochlorines and TAG were not in equilibrium. For example, Kelly and Campbell (1994) reported negative correlations between organochlorine and cod liver lipids. The authors attributed this result to a nonequilibrium situation in which lipid levels have changed more rapidly than the contaminant load. Lipophilic contaminants like PCBs and DDTs are associated with tissues with higher lipid (TAG) content, because they get partitioned there when they enter the body. However, lipid levels fluctuate. Since TAG is a short-term storage lipid, it mobilizes readily for reproductive purposes as well as energy requirements of the fish. It is believed that TAGs produced in the liver, but stored elsewhere, are packaged into lipoproteins for transport and are circulated as very low-density lipoproteins (Mommsen 1998). The transfer of organochlorines out of the liver would then depend on the binding compatibility between the organochlorine molecule and the lipoprotein (in

Table 2. Description of fish characteristics and organochlorine concentrations measured. Sample size = 10 for both species.

	California scorpionfish (*Scorpaena guttata*)				longfin sanddab *(Citharichthys xanthostigma)*			
Variable	Min.	Max.	Mean	95%CI	Min.	Max.	Mean	95%CI
Age (years)	2	4	3	0.5	2	5	4	1
Standard Length (mm)	165	212	194	11	113	150	131	7
Body Weight (g)	152	358	265	47	26	62	44	7
Condition	3.03	4.41	3.57	0.27	1.69	2.31	1.94	0.12
Liver (g)	3.1	12.9	6.6	1.8	0.6	1.5	1.0	0.2
Hepatosomatic Index	1.7	4.1	2.5	0.5	1.5	2.9	2.3	0.3
Organochlorines (µg/kg)								
Liver								
Total DDT	207	628	363	84	368	1,005	700	139
Total PCB	83	432	181	59	301	2,976	1,223	558
PCB118	nd	48	22	11	32	343	132	63
PCB138	nd	87	27	17	71	685	274	126
PCB153	46	134	66	16	87	855	369	152
PCB180	nd	77	22	17	62	304	160	55
PCB187	nd	65	22	14	46	285	147	54
Muscle								
Total DDT	nd	25	8	5	3	14	6	2
Lipid (percent wet weight)								
Liver								
Total Lipid	7.8	45.3	15.6	7.2	12.6	44.7	27.5	7.4
TAG*	3.3	29.2	8.2	5.0	5.9	40.6	22.0	8.2
PL*	2.8	16.9	5.7	2.7	2.1	7.6	4.9	1.2
Muscle								
Total Lipid	0.9	6.8	1.9	1.2	1.0	5.1	1.8	0.8
TAG*	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
PL*	0.8	6.1	1.8	1.0	1.0	4.8	1.6	0.7

*TAG = Triacylglycerols, PL = Phospholipids.

addition to whatever amount can be metabolized). If the affinity of the organochlorine to the lipoproteins is substantially lower than the affinity the organochlorine has for TAG (very likely, due to their hydrophobic nature), then the organochlorine remains in the liver, and therefore will not correlate with TAG.

The results from this study have implications for the practice of normalizing to lipid content. To account for variables such as species, age, size, and reproductive status, it has become common practice to facilitate the analysis of bioaccumulation data by normalizing for lipid content. However, if the relationship between lipid and contaminant concentration is not linear, then the process of normalization may result in the loss of data (Hebert and Keenleyside 1995). Because the lipid content in the livers and muscles of the scorpionfish and longfin sanddab did not correlate with the amount of organochlorine present, it would be inappropriate to normalize DDT and PCB concentrations to the amount of total lipid present.

Table 3. Regression equation r^2 and *p* values for relationship between lipid and organochlorines in liver and muscle tissue from longfin sanddab (*Citharichthys xanthostigma*) and California scorpionfish (*Scorpaena guttata*) collected off San Diego, California.

Model	Parameter	r^2	*p*	Model	Parameter	r^2	*p*
longfin sanddab				**California scorpionfish**			
Tissue/Lipid Type				**Tissue/Lipid Type**			
Liver/TAG*	tDDT	0.313	0.093	Liver/TAG*	tDDT	0.001	0.942
	tPCB	0.274	0.121		tPCB	0.002	0.907
	PCB118	0.255	0.137		PCB118	0.252	0.140
	PCB138	0.253	0.139		PCB138	0.050	0.534
	PCB153	0.246	0.145		PCB153	0.009	0.790
	PCB180	0.223	0.168		PCB180	0.000	0.969
	PCB187	0.250	0.141		PCB187	0.129	0.309
Muscle/TL*	tDDT	0.027	0.647	Muscle/TL*	tDDT	0.053	0.534

*TAG, triacylglycerols; TL, total lipid.
** tDDT, total DDT; tPCB, total PCB.

Literature Cited

Barron, M.G. 1990. Bioconcentration. *Environmental Science and Technology* 24: 1612-1618.

Bligh, E.G., and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911-917.

City of San Diego (COSD). 1998. Annual Receiving Waters Monitoring Report for the Point Loma Ocean Outfall, 1997. City of San Diego Ocean Monitoring Program, Metropolitan Wastewater Department, Environmental Monitoring and Technical Services Division. San Diego, CA.

City of San Diego (COSD). 2001. Annual Receiving Waters Monitoring Report for the Point Loma Ocean Outfall, 2000. City of San Diego Ocean Monitoring Program, Metropolitan Wastewater Department, Environmental Monitoring and Technical Services Division. San Diego, CA.

Ewald, G., and P. Larsson. 1994. Partitioning of polychorinated biphenyls between water and fish lipids. *Environmental Toxicology and Chemistry* 13: 1577-1580.

Groce, A.K. 2002. Influence of life history and lipids on the bioaccumulation of organochlorines in demersal fishes. M. Sc. Thesis. Department of Biological Sciences, San Diego State University, San Diego, CA.

Gutenmann, W.H., J.G. Ebel Jr., H.T. Kunts, K.S. Yourstone, and D.J. Lisk. 1992. Residues of p,p-DDE and mercury in lake trout as a function of age. *Archives of Environmental Contamination and Toxicology* 22: 452-455.

Hebert, C., and K.A. Keenleyside. 1995. To normalize or not to normalize? Fat is the question. *Environmental Toxicology and Chemistry* 14: 801-807.

Henderson, R.J. and D.R. Tocher. 1987. The lipid composition and biochemistry of freshwater fish. *Progress in Lipid Research* 26: 281-347.

Kelly, A.G., and L.A. Campbell. 1994. Organochlorine contaminants in liver of cod (*Gadus morhua*) and muscle of herring (*Clupea harengus*) from Scottish waters. *Marine Pollution Bulletin* 28: 103-108.

Love, M.S., B. Axell, P. Morris, R. Collins, and A. Brooks. 1987. Life history and fishery of the California scorpionfish, *Scorpaena guttata*, within the Southern California Bight. *Fisheries Bulletin* 85: 99-116.

Lovern, J.A. 1964. The lipids of marine organisms. *Oceanography and Marine Biology Annual Review* 2: 168-191.

Mann, R.A. and P. Ajani. 1991. Bioaccumulation of organochlorines and trace metals in fish-Sydney Deepwater Outfalls, Environmental Monitoring Program. pp. 65-70 *in*: A.G. Miskiewicz (ed.), Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts, and Bioaccumulation of Contaminants in Aquatic Environments. Australian Marine

Science Association, Inc./WaterBoard. Sydney, New South Wales.

Mommsen, T.P. 1998. Chapter 4: Growth and metabolism. pp. 65-73 in: D.H. Evans (ed.), *The Physiology of Fishes*. 2nd edition. CRC Press. Boca Raton, FL.

Nichols, P.D., M.J. Bakes and N.G. Elliot. 1998. Docosahexaenoic acid-rich liver oils from temperate Australian sharks. *Marine and Freshwater Research* 49: 763-767.

Nowak, B. 1991. Experimental investigation of endosulfan residues in tissues of catfish (*Tandanus tandanus*). pp. 213-224 *in:* A.G. Miskiewicz (ed.), Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts, and Bioaccumulation of Contaminants in Aquatic Environments. Australian Marine Science Association, Inc./WaterBoard. Sydney, New South Wales.

Otway, N. 1991. Bioaccumulation studies on fish: Choice of species, sampling designs, problems and implications for environmental management. pp. 103-113 *in:* A. G. Miskiewicz (ed.), Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts, and Bioaccumulation of Contaminants in Aquatic Environments. Australian Marine Science Association, Inc./WaterBoard. Sydney, New South Wales.

Pastor, D., J. Boix, V. Fernandez and J. Albaiges. 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus, Mugil cephalus*, and *Dicentrarcus labrax*). *Marine Pollution Bulletin* 32: 257-262.

Phillips, D.J.H. 1991. The bioaccumulation of trace contaminants in aquatic environments: A review. pp. 305-322 *in:* A.G. Miskiewicz (ed.), Proceedings of a Bioaccumulation Workshop: Assessment of the Distribution, Impacts, and Bioaccumulation of Contaminants in Aquatic Environments. Australian Marine Science Association, Inc./WaterBoard. Sydney, New South Wales.

Phillips, D.J.H. 1995. The chemistries and environmental fates of trace metals and organochlorines in aquatic ecosystems. *Marine Pollution Bulletin* 31: 193-200.

Swackhammer, D.L. and R.A. Hites. 1988. Occurrence and bioaccumulation of organochlorine compounds in fish from Siskiwit Lake, Isle Royale, Lake Superior. *Environmental Science and Technology* 22: 543-548.

Van Wezel, A.P. and A. Opperhuizen. 1995. Thermodynamics of partitioning of a series of chlorobenzenes to fish storage lipids, in comparison to partitioning to phospholipids. *Chemosphere* 31: 3605-3615.

Volkman, J.K. and P.D. Nichols. 1991. Applications of thin layer chromatography-flame ionization detection to the analysis of lipids and pollutants in marine environmental samples. Journal of Planar Chromatography 4: 19-26.

Acknowledgements

This study was conducted as part of the first author's Master of Science thesis for San Diego State University (Groce 2002). The authors thank Dr. M. Ohman, who provided space, equipment, and tremendous assistance for the lipid research. We also thank D. Young and D. Tsukada, for their assistance with the organochlorine analysis. The authors thank R. Gartman, S. Lagos, R. Duggan, E. Nestler, and M. Kelly for their help with the collection of specimens. They also thank A. Langworthy, L. Vereker, P. Vainik, T. Stebbins, and L. Wiborg for laboratory support.