Chemical Contamination and the Annual Summer Die-Off of Striped Bass (*Morone saxatilis*) in the Sacramento–San Joaquin Delta

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In 1987, striped bass (*Morone saxatilis*) that were nearly dead (moribund) were captured by hand net, and apparently healthy striped bass were caught by hook and line from adjacent waters in the Sacramento–San Joaquin Delta or, alternatively, caught by hook and line from the Pacific Ocean. The livers of these three groups of striped bass were examined for chemical contamination by gas chromatography, by gas chromatography–mass spectrometry, and by immunoassay. Moribund striped bass livers were greatly contaminated by chemicals compared to healthy fish caught in the Delta and the Pacific Ocean. The types of contaminant encountered suggested that industrial, agricultural, and urban pollutants were present in the livers of moribund fish. Although the variability in the amount of hepatic contaminants observed among the groups of fish does not provide direct proof of causation, the large amount of pollutants suggests that chemical contamination (possibly acting as multiple stressors) contributes to the hepatotoxic condition of the moribund striped bass and may lead to an explanation of the die-off in the Sacramento–San Joaquin Delta region.

The San Francisco Bay and the Sacramento–San Joaquin Delta together comprise the largest estuary in the Western United States; the estuary covers approximately 1600 square miles and drains over 40% of the state. One of the most important species of economic and ecological value residing in the Delta is the striped bass, *Morone saxatilis*, first introduced into the Delta and established as a thriving fishery in the late 1800s (*1*). Since the mid-1970s the striped bass population has experienced an alarming decline, and larval recruitment has been extremely low. Today, the striped bass population in the estuary is at an all-time low.[1] Presumably, contributing to the decline are factors such as (a) reduced adult stock producing fewer eggs, (b) reduced food production in the upper Delta, (c) loss of larval fish into water diversion projects, and (d) toxic effects of water-borne pollutants (*2*). The striped bass die-off largely occurs during the summer months when hundreds to thousands of dead adult striped bass are found, most often in the Carquinez Straits area (*3*). The most obvious pathological aspect of moribund striped bass is liver dysfunction (*4*, *5*), although various endocrine glands as well as the kidney and intestine are damaged. The seasonal striped bass die-off coincides with the discharge of several herbicides used in the cultivation of rice, although other agricultural, industrial, and urban chemicals are also present in the Delta (*6*). Urquhart and Knudsen report that striped bass collected from the Delta have been shown to contain hydrocarbons, chlorinated hydrocarbons, and heavy metals.[1] In striped bass which were taken from the San Joaquin or Sacramento Rivers, DDT levels were 16 and 31 ng/g of liver, respectively. Alicyclic hexane levels in striped bass collected in the San Joaquin River were 10 ng/g of liver. No detectable amount of alicyclic hexanes was observed in the liver of striped bass taken from the Sacramento River. Aroclor 1260, a mixture of polychlorinated biphenyls, was present in the liver of striped bass taken from the San Joaquin or Sacramento Rivers at concentrations of 440 and 760 ng/g of liver, respectively.[1] A general correlation between hepatic chemical contaminants and striped bass health has been reported (*2*). The definitive cause of the striped bass die-off remains uncertain, although chemical contamination has been proposed as a possible explanation (*2*).

In this report, the chemical contaminants present in the livers of moribund and "healthy" striped bass were examined and compared with those occurring in the livers of adult fish caught in the Pacific Ocean. Knowledge of the chemical contaminants present in striped bass could provide insight into the cause of the striped bass die-off, as well as indicate the type and quantity of pollutants present in the San Francisco Bay estuary that may have direct effects on human health. The occurrence and demonstration of exposure, body burdens, and toxic effects of agricultural and environmental chemicals in the liver of striped bass may also have implications for the food and fish industries.

Materials and Methods

Animals. In late June 1987, both juvenile and adult moribund striped bass were captured for laboratory analyses for the first time from the Carquinez Strait. Moribund striped bass that were

[1] K. A. F. Urguhart and D. L. Knudsen (1987) Striped bass health monitoring final report. California Department of Fish and Game, California State Water Resources and Control Board, Stockton, CA 95205 (unpublished report).

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Table I. Physical Characteristics of Ocean Control, Delta Control, and Moribund Striped Bass

	ocean control[a]		Delta control[b]		moribund[c]	
fish no.	weight, lb	length, in.	weight, lb	length, in.	weight, lb	length, in.
1	12.0	29.3	2.5	17.5	7.8	26.5
2	6.0	25.0	1.5	13.5	2.8	18.0
3	24.0	40.5	2.8	18.5	2.5	17.0
4	5.0	23.5	2.0	16.3	1.8	14.5
5	18.0	37.0	2.0	16.0	1.8	13.0
6	10.0	30.5	3.8	20.5	5.5	24.5
7	4.0	24.0	7.8	26.5	3.8	19
8	8.0	26.0	2.3	17.0	8.3	27

[a] All of the fish were mature adults, and there were 4 males and 4 females. [b] The larger fish were postspawning females, others were immature, and the remainder were in a sex ratio of 50:50. [c] The sex of fish 1–3 was nondeterminable, fish 4 was female (gravid), fish 5 and 7 were immature males, fish 6 was a mature female, and 8 was a female.

swimming passively with the current on or near the water surface appeared oblivious to potential danger and were easily captured with a landing net. Moribund fish were landed on the shore within a few minutes and were immediately killed. At the same time, Delta control fish were captured by hook and line in the same general area of the Carquinez Strait. Mature fish were caught by hook and line in the Pacific Ocean off Pacifica (in 1988) and served as "ocean control" fish (*4*). The physical characteristics of the fish examined are presented in Table I. It should be pointed out that the moribund population represents a severely impaired group of fish that survived in spite of many possible processes of elimination. Although this field sample is somewhat heterogeneous with regard to age and sex, the moribund fish represent a rare group that may simply not be often obtainable but nevertheless is a significant biological indicator of pollution.

Aside from the yellowish tinge of varying degree of their ventral surface, moribund striped bass appeared outwardly normal. Upon dissection, several differences between moribund and healthy Carquinez controls were apparent. The livers of approximately 75% of the moribund fish were mottled and discolored, with evidence of localized hemorrhage in about 25% of these. Only 12% Carquinez control and 13% of ocean control fish showed liver mottling and discoloration; in all other respects control fish appeared normal. Because of the obvious hepatic necrosis we examined livers of striped bass for the presence of potentially hepatotoxic materials because others have demonstrated a correlation between hepatic pollutant burden and pathology of striped bass (*2*). In contrast to control fish, the kidney, intestine, thyroid, and interrenal tissue of moribund fish exhibited a range of pathological symptoms, and these observations will be reported elsewhere (*5*).

Chemicals. Chemicals used in this study were of the highest purity available and were purchased from Aldrich Chemical, Milwaukee, WI. Other reagents, buffers, and solvents were from Fisher Scientific, San Francisco, CA. Molinate was a gift from Stauffer Chemical Co., Richmond, CA. Thiobencarb was a gift of Chevron Chemical Co., Richmond, CA. Atrazine was a gift of Ciba-Geigy, Greensboro, NC. The other chemical standards were purchased from Chemical Service, West Chester, PA.

Reagents. Immunochemicals were purchased from Sigma Chemical Co. (St. Louis, MO) or ICN Immunobiologicals (Lisle, IL). The production of herbicide haptens, hapten–protein conjugates, and rabbit anti-herbicide antibodies has been described previously (*7–9*). Thin-layer chromatography plates (LK5DF) were obtained from Fisher Scientific.

Preparation of Striped Bass Hepatic Extracts for Chemical Analysis. Frozen striped bass livers (1.0–1.6 g) obtained as described (*5*) were minced into small pieces with a razor blade on a cold glass plate, placed in a cold glass test tube with 12 mL of aqueous acetonitrile (1:1 v:v), and homogenized with a Branson sonicator until the tissue was completely disrupted. The homogenate was stirred for 30 min at room temperature and then centrifuged (2000*g*, 10 min). The supernatant was decanted, and 2 mL of brine and 2 mL of hexane were added and mixed thoroughly; the organic layer was separated by centrifugation and saved for analysis (nonpolar low-volatility fraction). Separate pieces of liver were chopped into fine pieces, homogenized in cold aqueous acetonitrile (1:1 v:v), and centrifuged at 2000*g* for 10 min. The supernatant was decanted and sequentially extracted with ethyl acetate, $CHCl_3$, sodium borate/CH_3CN (1:1 v:v), oxalic acid/CH_3CN (1:1 v:v), and finally CH_3CN. The organic extracts were combined, washed with hexane, and evaporated to dryness (polar low-volatility fraction). A portion of the residue was dissolved in benzene, placed on a LK5DF thin-layer chromatography (TLC)[2] plate, and developed with ethyl acetate/hexane (20:80 v:v). After development of the TLC plate, three regions were found to be heavily stained by the spray reagent phosphomolybdic acid with R_f values of 0.8, 0.4, and 0.1–0.2. Each fraction was further evaluated by gas chromatography, and selected samples were analyzed by gas chromatography–mass spectrometry.

Chromatographic Analysis of Nonpolar and Polar Low-Volatility Hepatic Extracts. All gas chromatographic runs were carried out on a Hewlett-Packard Model 5890 gas chromatograph equipped with thermal conductivity detectors. A capillary column (30 m × 0.25 μm i.d.) packed with DB-1 was used. The carrier gas was helium. The linear temperature program was started at 40 °C isothermally for 3 min, after which time the temperature increased to 200 °C at a rate of 8 °C/min and was maintained at 200 °C isothermally for 1 min; the temperature was then increased to 300 °C at a rate of 5 °C/min. The injection port and the detector were kept at 300 °C. Standard references of *n*-alkanes (C_5–C_{22}) and/or dialkyl phthalates (dimethyl–dinonyl) were injected each time a series of samples was run. The retention index (I) of the chemicals was computed by employing the equation described by Peng et al. (*10*):

$$I = 100i\frac{X - M_{(n)}}{M_{(n+i)} - M_{(n)}} + 100n$$

where n is the number of carbon atoms in the *n*-alkane standard and X, $M_{(n)}$, and $M_{(n+i)}$ are the adjusted retention times (corrected for the air peak) of the sample, $M_{(n)}$ is the alkane marker with n carbon atoms eluting before the sample, and $M_{(n+i)}$ is the alkane with $(n + i)$ carbon atoms eluting after the sample.

The amount of material quantified in each chromatogram was calculated from external calibration curves. In addition, retention index analysis with authentic standards (described above) gave approximate molecular weights and predicted structures that were later confirmed by gas chromatography–mass spectroscopy. Standard curves for *n*-alkanes, dialkyl phthalates, and selected herbicides were linear from 1 to 100 ng. Amounts of unknown were calculated from the areas of the gas chromatography peaks. Under the conditions of the runs, the chromatograms were highly reproducible and the limit of detection was 1 ng.

Gas Chromatography–Mass Spectrometry. Gas chromatography–mass spectra were obtained with a VG70S spectrometer fitted with a Varian Model 3600 gas chromatograph and a DB-1 capillary column (30 m × 0.25 μm i.d.). The carrier gas was helium. The linear temperature program was started at 80 °C and increased to 300 °C at a rate of 4 °C/min. High-resolution gas chromatography–mass spectrometry was performed by peak matching with perfluorinated hydrocarbons and other standards.

Analysis of Herbicides by Immunosorbent Assay. ELISA and competitive inhibition ELISA were performed (*8*, *9*) in 96-well microplates (Nunc Maxisorb Roskilde, DK no. 442404). Samples of striped bass liver (average weight 3.1 g) were homogenized in 24 mL of cold acetonitrile/water (15:85 v:v) using a homogenizer (Brinkman Instruments) and centrifuged at 10000*g* for 20 min. Homogenates were stored at 4 °C until the following day. A C-8 solid-phase extraction column (Analytichem, Harbor City, CA) was used for extraction and concentration of the sample. The column was prepared prior to sample application by washing with 3 mL of acetonitrile and then two times with 3 mL of glass-distilled water. Homogenate supernatants (2 mL) were drawn through the C-8 columns by a Baker SPE vacuum manifold. The sample was eluted with two 2-mL volumes of methanol. Methanol eluates were stored in Teflon-capped glass vials at −20 °C until analysis.

[2] Abbreviations: TLC, thin-layer chromatography; ELISA, enzyme-linked immunosorbent assay; GC–MS, gas chromatography–mass spectrometry; BCF, bioconcentration factor.

ELISA. A haptenated enzyme format similar to that previously reported (*11*) was used for the analysis of molinate and triazines. The haptenated enzyme format was as follows. On the day of the experiment, a competition plate was prepared in which 40 μL of each sample, 100 μL of haptenated enzyme, and 100 μL of herbicide-specific antibody were added to each well and incubated for 2 h. A separate 96-well polystyrene microtiter plate (Nunc Maxisorb) was coated with a 1/1000 dilution of sheep anti-rabbit IgG (100 μL/well) overnight at 4 °C. This coated plate then was washed 5 times with 0.05% Tween 20 in water. The plates were treated with 1% bovine serum albumin (100 μL/well for 1 h) for blocking. Following blocking, the IgG-coated plate was washed, and 50 μL from each well of the competition plate was added to the IgG-coated plate. The plate was incubated for 2 h at room temperature. The plate was washed and substrate was added, the mixture was incubated for 45 min, 50 μL of sulfuric acid were added, and the color development was read at 450 nm with a V_{max} microplate reader (Molecular Devices, Menlo Park, CA). All inhibition curves used for calculation of IC_{50} values were composed of zero-dose control plus 10 nonzero standard concentrations, with quadruplicate ELISA wells at each concentration. The software package Softmax (v. 2.01, Molecular Devices) was used for fitting standard curves according to the method of Rodbard (*12*).

For the analysis of triazines, the above format was followed except that the haptenated tracer was alkaline phosphatase, the substrate was *p*-nitrophenyl phosphate, and the resulting color was read at 405 nm.

For thiobencarb, an antigen-coated plate format was used exactly as described by Gee et al. (*7*) for molinate. Briefly, a 96-well plate was coated with a thiobencarb hapten coupled to protein. The sample was preincubated with an anti-thiobencarb antibody. The antibody–sample mixture was then incubated on the coated plate. After exhaustive washing with PBS–Tween azide, a goat anti-rabbit IgG coupled to alkaline phosphatase was added. After incubation and washing of the plate, the substrate, *p*-nitrophenyl phosphate, was added and the resulting absorption was read at 405 nm (*7*).

Statistical Analysis. Statistical analyses were done using a one-way analysis of variance and the multiple-range test developed by Duncan (*13*).

Results

The livers of striped bass obtained by hook and line in late June 1988 from the Pacific Ocean (ocean controls) and the Carquinez Straits in 1987 (Delta controls), as well as those of moribund striped bass from the Carquinez Straits, were homogenized and extracted with organic solvents. The analysis of nonpolar low-volatility materials obtained from hexane extracts was greatly assisted by our use of retention index relationships (*10*). Thus, gas chromatography of a series of aliphatic alkanes on apolar columns obeyed a retention index–molecular weight correlation in much the same manner as the nonpolar low-volatility materials observed in the livers of moribund striped bass (Table II). Comparison of aliphatic hydrocarbon standard retention indices with unknowns from striped bass livers provided a general method to predict the structure and molecular weight of the unknowns. The identity of the unknown was confirmed by gas chromatography–mass spectrometry (GC–MS), and in some cases, the molecular formula was confirmed by high-resolution GC–MS. Table II showed that livers from ocean control and Delta control striped bass contained significant levels of relatively low molecular weight hydrocarbons (e.g., **1–3**). Livers from moribund striped bass did not contain high levels of low molecular weight hydrocarbons, but rather contained higher molecular weight hydrocarbons and waxes (e.g., **5, 6, 8–13, 15–19**) as well as materials containing one oxygen atom (e.g., **4, 7, 14**).

The mass spectra of **4, 7,** and **14** were determined by GC–MS and revealed interesting fragmentation patterns. Because of the large number of structures possible we were unable to unambiguously identify the compounds. Several conclusions were deduced, however: (a) the materials were structurally related to one another, (b) the materials contained aromatic and aliphatic moieties, and (c) a nonalcoholic oxygen was present in the molecules. For **4**, the GC–MS gave prominent ions of *m/z* (relative abundance) 268 (34), 253 (100), 237 (6), 191 (9), 91 (20), 77 (9). For **7**, the GC–MS gave prominent ions of *m/z* (relative abundance) 324 (68), 309 (100), 293 (14), 247 (15), 91 (47), 77 (12). For **14**, the GC–MS gave prominent ions of *m/z* (relative abundance) 448 (68), 433 (100), 355 (20), 277 (5), 117 (39), 91 (39), 77 (9).

Table II. Nonpolar Low-Volatility Chemical Contaminants Detected in the Liver of Striped Bass[a]

compd no.	molecular formula	μg/g of liver		
		ocean control	Delta control	moribund
1	$C_{16}H_{34}$	0.1	0.1	0.2
2	$C_{18}H_{38}$	1.9	0.7	ND[b]
3	$C_{19}H_{40}$	0.2	0.2	0.5
4	$C_{19}H_{24}O_1$	ND	ND	1.8[c]
5	$C_{21}H_{44}$	0.03	ND	0.3
6	$C_{22}H_{46}$	0.1	0.2	1.8
7	$C_{23}H_{32}O_1$	ND	ND	2.4[c]
8	$C_{23}H_{48}$	ND	ND	2.4
9	$C_{24}H_{50}$	ND	ND	2.7
10	$C_{26}H_{54}$	ND	ND	2.4
11	$C_{27}H_{56}$	ND	ND	1.8
12	$C_{28}H_{58}$	ND	ND	1.5
13	$C_{29}H_{60}$	ND	ND	1.7
14	$C_{33}H_{36}O_1$	ND	ND	0.8[c]
15	$C_{30}H_{62}$	ND	ND	0.5
16	$C_{31}H_{64}$	ND	ND	0.4
17	$C_{32}H_{66}$	ND	ND	0.2
18	$C_{33}H_{68}$	ND	ND	0.2
19	$C_{34}H_{70}$	ND	ND	0.3

[a] Results presented as the mean for 2–3 determinations by gas chromatography and confirmed by gas chromatography–mass spectrometry (i.e., molecular ion and fragmentation pattern) as described in Materials and Methods. [b] ND, not detected (<0.01 μg/g of liver). [c] Molecular formula confirmed by high-resolution gas chromatography–mass spectrometry.

Separate pieces of liver were chopped into fine pieces, homogenized, and centrifuged; the supernatant was decanted and sequentially extracted as described above. Table III lists the major chemicals (R_f values 0.4–0.8) isolated from TLC. The large amounts of various dialkyl phthalates present in the livers of moribund striped bass were absent (**25–28**) or present only as minor materials (**20–24**) in the livers of control striped bass (Table III). Dialkyl phthalates have been observed in water analyzed from the Delta, but the amounts observed in the liver of moribund striped bass were considerably greater than those reported to be present in Delta water.[3] The concentration of dialkyl phthalates **20, 22,** and **26** present in industrial waste water discharged into the Delta was reported to be 1–100 μg/L. The ambient dialkyl phthalate concentration estimated or observed in the San Francisco Bay region was zero. In July 1990, however, dialkyl phthalates were detected in the water of the Carquinez Straits,[4] and in the winter of 1986 dialkyl phthalates were detected in San Francisco Bay sediment (*14*). That the dialkyl phthalates arose from environmental contamination and not from sample preparation was seen from the large amount of material detected, and from the number of polar

[3] W. Pease, K. Taylor, and S. Anderson (1989) Toxic substances of concern and potential water quality objectives for the protection of aquatic life and human health, San Francisco Bay Regional Water Quality Control Board, Oakland, CA 94607 (unpublished report).

[4] W. Perera, Ph.D., USGS, Menlo Park, CA, personal communication.

Table III. Dialkyl Phthalates Detected in the Liver of Striped Bass[a]

compd no.	dialkyl phthalate	μg/g of liver			statistical comparisons[b]
		ocean control	Delta control	moribund	
20	diethyl	83.9 ± 35.3	84.4 ± 23.4	183.9 ± 160.9	OCM
21	dibutyl	3.5 ± 1.4	8.0 ± 5.3	11.7 ± 1.2	OCM
22	diamyl	1.1 ± 1.6	9.8 ± 10.7	24.4 ± 5.2	OCM
23	bis(ethylhexyl)	2.8 ± 2.2	3.7 ± 2.6	13.9 ± 9.5	OCM
24	diisohexyl	4.2 ± 3.0	5.5 ± 3.9	52.5 ± 6.1	OCM
25	diisoheptyl	0 ± 0	1.0 ± 2.5	13.8 ± 5.4	OCM
26	dioctyl	0.3 ± 0.6	3.2 ± 3.6	20.9 ± 19.3	OCM
27	dinonyl	1.5 ± 1.2	3.2 ± 3.6	33.0 ± 62.2	OCM
28	didecyl	0 ± 0	9.0 ± 15.2	27.0 ± 49.5	OCM

[a] Results presented as mean ± SD, $n = 6$, for each group determined by gas chromatography and confirmed by gas chromatography–mass spectrometry as described in Materials and Methods. [b] Statistical comparisons: O, ocean control; C, Carquinez control; M, moribund. Groups are arranged left to right in the order of descending magnitude for each treatment. Groups jointly underlined are not significantly different ($p > 0.05$) from each other. Statistical analyses were done using a one-way analysis of variance and Duncan's multiple-range test (*13*).

materials (R_f 0.1–0.2) that we also observed by GC–MS, presumably arising from degradation of dialkyl phthalates, including hexanediol, decanoic acid, octadecanoic acid, hexadecanoic acid, and benzoic acid. Thus, hexanediol had prominent ions at m/z (relative abundance) 115 (12), 82 (21), 70 (40), 67 (76), 57 (55), 42 (100); decanoic acid had prominent ions at m/z (relative abundance) 172 (8), 129 (64), 103 (71), 85 (81), 71 (100); octadecanoic acid had prominent ions at m/z (relative abundance) 284 (57), 241 (34), 185 (28), 129 (67), 73 (76), 60 (100); hexadecanoic acid had prominent ions at m/z (relative abundance) 256 (73), 213 (52), 167 (38), 129 (85), 60 (100); and benzoic acid had prominent ions at m/z (relative abundance) 122 (84), 105 (100), 77 (64), 51 (24). The spectra of the identified materials were essentially identical to mass spectral library reference spectra. In addition, other polar chemicals were detected in the liver of moribund striped bass by GC–MS: benzothiazole, tetradecanoic acid, tartaric acid diethyl ester, and 9-octadecanoic acid (2,3-dihydroxypropyl ester). Thus, benzothiazole had prominent ions at m/z (relative abundance) 135 (100), 108 (89), 91 (22), 82 (30), 69 (67); tetradecanoic acid had prominent ions at m/z (relative abundance) 228 (22), 185 (31), 129 (51), 73 (100), 55 (96); tartaric acid diethyl ester had prominent ions at m/z (relative abundance) 133 (75), 104 (100), 87 (25), 76 (95), 59 (26) (no molecular ion); and 9-octadecanoic acid (2,3-dihydroxypropyl ester) had prominent ions at m/z (relative abundance) 356 (12) 338 (44), 325 (22) 264 (37), 98 (51), 69 (67), 55 (100). The spectra of the identified materials were essentially identical to mass spectral library reference spectra.

Due to the considerable use of herbicides in rice paddies occurring in the Sacramento–San Joaquin Delta (*6*), we determined the amount of herbicides present in the liver of striped bass. We analyzed striped bass liver homogenates for molinate and thiobencarb. Because some of the herbicides are volatile and thus apt to be present in low amounts compared to dialkyl phthalates and hydrocarbon materials, analyses were performed with an ELISA-based assay (*7–9*). The advantages of these enzyme-linked immunosorbent assays (ELISA) lie in their sensitivity and their ability to analyze samples directly without extraction, evaporation, or concentration steps (*15*). Because triazine herbicides are among the most common compounds found present in both surface and ground water contaminated by agricultural chemicals in North America (*16*), we analyzed striped bass liver for triazines for comparison with the other class of herbicides. As shown in Table IV, considerable amounts of immunoreactive materials were present in the liver of moribund striped bass based on analysis with herbicide-selective

Table IV. Herbicides Detected in the Liver of Striped Bass[a]

compd no.	herbicide	ng/g of liver		
		ocean control	Delta control	moribund
29	triazines	56.1 ± 29.5[b]	23.1 ± 14.2	52.1 ± 8.0
30	molinate	79.1 ± 39.0	42.0 ± 31.2	63.1 ± 62.4
31	thiobencarb	495.5 ± 641.3[c]	630.5 ± 746.2[c]	1190.2 ± 1403.2

[a] Results determined by ELISA presented as mean ± SD, $n = 7$. As a control, offspring of pregnant striped bass captured in the Carquinez Straights area (Delta controls) were raised in captivity, and herbicides were determined by ELISA. The values of triazines, molinate, and thiobencarb were 14, 34, and 197 ng/g of liver, respectively, $n = 1$. [b] Statistical analyses were done using a one-way analysis of variance and Duncan's multiple-range test (*13*). None of the groups was significantly different ($p > 0.05$) from the others. [c] Results determined by ELISA presented as mean ± SD, $n = 4$.

antibodies. There was no statistically significant relation among the amount of immunoreactive material in the liver and the sex or maturity status of the fish examined. However, there was a tendency for larger fish to have more herbicide present in the liver, and interestingly, the highest amount of immunoreactive material to the anti-thiobencarb antibody was found in the liver of a pregnant fish. Overall, the data showed considerable variation (e.g., **30**, **31**); however, large differences in the mean values were nevertheless obvious. Attempts to confirm the presence of thiobencarb in the liver extracts of moribund striped bass by GC–MS were confounded by the high levels of dibutyl phthalate which cochromatographed with thiobencarb in our gas chromatography system. These interesting observations should be confirmed by independent analysis or by showing that the immunoreactive material has the same retention volume as the parent herbicide or metabolite on HPLC.

Discussion

Within the past 20 years, evidence of agricultural, industrial, and urban pollution in the Delta has increased (*2*, *6*). Aside from metal contaminants, such as arsenic, aluminum, copper, iron, magnesium, and selenium (*17*), most of the pollutants reported are highly lipophilic and mainly distributed in biota, sediment, and other lipophilic domains. For example, poorly water-soluble thiobencarb and dialkyl phthalates have log P (*18*) [log partition coefficient (octanol/H_2O)] values ≥2.5 (*19*) and should mainly partition into biota and sediment. The log P values for dialkyl phthalates range from 2.56 to 11.2 for **20–28**, respectively. The calculated logarithm of the bioconcentration factor (BCF) (*20*) for each dialkyl phthalate parallels the log P value (i.e., BCF for **20** and **28** are 1.55 and 8.45, respectively) according to log BCF = 0.79 log P − 0.4. Similarly, the log P values for atrazine, molinate, and

thiobencarb are 2.75, 3.21, and 3.4, and the logarithms of the BCF are 1.77, 2.14, and 2.29, respectively. These calculations[5] show that significant accumulation of **20–31** should be observed, and this was the case. While the mechanism of transport of the lipophilic materials into the fish is unknown, it is unlikely that the lipophilic pollutants are being directly transported into the fish via the water. It is possible that the material is transported into moribund striped bass via sediment or benthic sources.

The bioconcentration factors for thiobencarb in striped bass reported previously have ranged between 20 and 100 (*6*). Moribund striped bass may be able to bioconcentrate pollutants to a much greater extent than previously recognized. It is possible that lipophillic compounds might partition better into the livers of moribund compared to control stripped bass due to the amount of paraffins present in the livers of moribund striped bass. The variation in the amounts of immunoreactive material to herbicide-selective antibodies detected in the liver of moribund striped bass may reflect the fact that the sample of fish collected was quite heterogeneous. It is highly unlikely that methodological variation is responsible for the results. Nevertheless, it is important to report data on this rare field sample of moribund striped bass. The presence of detectable levels of thiobencarb and other herbicides used in the cultivation of rice present in Delta water (*6*) suggested that sufficient amounts of herbicides are present for accumulation into the tissue of striped bass and bioactivation to potent hepatotoxins (*21*, *22*). Our study suggests that lipophilic chemical pollutants should be assessed in biota and other lipophilic domains and that risk assessment of the toxicity of materials based on apparent concentrations in water underestimates the true chemical burden and hence potential toxicity to organisms. This may be especially true for herbicides such as thiobencarb and molinate which are efficiently biotransformed by striped bass (*21*, *23*, *24*). Previously, we have shown that thiocarbamate herbicides are extensively oxidized by hepatic microsomes prepared from striped bass. *S*-Oxide and sulfone metabolites of thiocarbamate herbicides are considerably more electrophilic than their parent thiocarbamate herbicides, and we have shown that the S-oxidized thiocarbamate metabolites are efficient carbamylating agents (*21*, *22*). The high levels of a large number of dialkyl phthalates, as well as putative dialkyl phthalate metabolites, observed in moribund striped bass and present in lower or negligible levels in Delta or ocean control striped bass suggest that the dialkyl phthalates do not come from laboratory contamination but originate from an unknown environmental source and consist of a large number of chemically diverse compounds. Among the possible sources of dialkyl phthalates are the ordinary garden hose and vinyl agricultural plumbing.[6] The ability of contaminated striped bass to "purge" themselves of chemical contaminants after returning to the ocean (i.e., depurination) following spawning can be seen from the reduced amount of chemicals in the liver of ocean control striped bass. It is possible that Delta control striped bass may not have absorbed the chemical contaminants to the same extent as moribund fish. Because dialkyl phthalates are efficiently hydrolyzed (*25*) and generally nontoxic in other fish (*26*) and mammals (*27*), the accumulation of significant amounts of intact dialkyl phthalates in moribund striped bass, compounds that may be readily detoxicated in other species, suggests that dialkyl phthalates may contribute to the observed hepatotoxicity of moribund striped bass. Other oxygen-containing hydrocarbons and aliphatic and aromatic hydrocarbons may be present in the liver of moribund striped bass at considerably lower levels than dialkyl phthalates (Table III), and the former may still also pose a significant hepatotoxic risk. Although the structures of **4**, **7**, and **14** could not be unambiguously identified, their mass spectral properties were consistent with those of oxygen-containing hydrocarbons. Mass spectral fragmentation patterns of **4**, **7**, and **14** all indicated loss of aliphatic and/or aromatic groups before loss of oxygen. No evidence for alcohol oxygen was observed, and we conclude that the oxygen is an internal part of the hydrocarbon structure. Such structures as alkylated phenols and related polycyclic and monocyclic aromatic hydrocarbons are not known to be hepatotoxic to striped bass (*28*, *29*), however.

In summary, moribund striped bass are heavily contaminated with a variety of industrial (e.g., aliphatic hydrocarbons, oxygen-containing hydrocarbons, aliphatic esters), agricultural (e.g., herbicide-like materials, stabilizers), and urban pollutants (e.g., benzothiazole, petroleum-based constituents, and dialkyl phthalates). The variability in the amounts of contaminants observed in the liver of striped bass does not allow direct conclusion as to the causative agents, but the large amount of pollutants clearly suggests that chemical contamination may contribute to the striped bass die-off in the Sacramento–San Joaquin Delta region, possibly as a result of multiple stressors.

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[5] Calculated by Dr. P. S. Magee, Biosar Research, Vallejo, CA.
[6] Leonard Curry, Richmond, CA, personal communication.

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