Relationships between Toxicopathic Hepatic Lesions and Exposure to Chemical Contaminants in English Sole (*Pleuronectes vetulus*), Starry Flounder (*Platichthys stellatus*), and White Croaker (*Genyonemus lineatus*) from Selected Marine Sites on the Pacific Coast, USA

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Relationships between hepatic lesions and chemical contaminant concentrations in sediments, stomach contents, liver tissue, and bile were statistically evaluated in three species of bottomfish, English sole (*Pleuronectes vetulus*), starry flounder (*Platichthys stellatus*), and white croaker (*Genyonemus lineatus*), captured from 27 urban and nonurban sites on the Pacific Coast from Alaska to southern California. Lesions detected were neoplasms, preneoplastic foci of cellular alteration, nonneoplastic proliferative lesions, unique or specific degenerative/necrotic lesions, nonspecific degenerative/necrotic lesions, and hydropic vacuolation of biliary epithelial cells and hepatocytes. In general, lesion prevalences were significantly higher in all three species captured at chemically contaminated urban sites, and certain lesions had significantly higher relative risks of occurrence at urban sites in Puget Sound, San Francisco Bay, the vicinity of Los Angeles, and San Diego Bay. Concentrations of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, DDT and its derivatives, and chlordanes and dieldrin in sediment, stomach contents, liver, and fluorescent aromatic compounds in bile were significant risk factors for the occurrence of neoplastic, preneoplastic, nonneoplastic proliferative, and specific degenerative/necrotic lesions, as well as hydropic vacuolation. Fish age also had a significant influence on occurrence of several hepatic lesions, but gender was rarely a significant risk factor. These relationships provide strong evidence for the involvement of environmental contaminants in the etiology of hepatic lesions in several marine bottomfish species and clearly indicate the utility of these lesions as biomarkers of contaminant-induced effects in wild fish. *Key words:* contaminant exposure, epizootiology, fish hepatic lesions, histopathology. *Environ Health Perspect* 102:200–215(1994)

Certain hepatic lesions found in wild fish, including neoplasms, preneoplastic focal lesions, other proliferative lesions, and degenerative/necrotic lesions resemble those induced by experimental exposure of fish (*1–7*) and rodents (*8–12*) to a variety of toxicants including carcinogens. These toxicopathic lesions have been positively associated with exposure to xenobiotic contaminants in numerous field studies on bottomfish species from marine and estuarine coastal areas. Affected species include English sole (*Pleuronectes vetulus*) (*13–21*), Atlantic tomcod (*Microgadus tomcod*) (*22,23*), white croaker (*Genyonemus lineatus*) (*13,20,24,25*), mummichog (*Fundulus heteroclitus*) (*26*), winter flounder (*Pleuronectes americanus*) (*27–32*), starry flounder (*Platichthys stellatus*) (*33,34*), European flounder (*Platichthys flesus*), common dab (*Limanda limanda*) (*35–37*), and others (*38*). Cause-and-effect relationships suggested from field surveys have been supported by statistical analyses (*15,19,20, 34,39,40*) and by long-term laboratory studies in which liver lesions identical to those observed in field-captured English sole (i.e., preneoplastic focal lesions, nonneoplastic proliferative lesions, megalocytic hepatosis) were induced by multiple injections of an organic-solvent extract from a sediment collected at a site heavily contaminated with polycyclic aromatic hydrocarbons (PAHs) (*3*); English sole from this site in Puget Sound are affected by high prevalences of toxicopathic hepatic lesions (*16,18*). Moreover, hepatocellular carcinomas were induced in rainbow trout (*Oncorhynchus mykiss*) as a surrogate species by microinjection of sac-fry embryos with an organic-solvent extract of chemically contaminated sediments from Hamilton Harbor, Ontario (*41,42*); the species at environmental risk in this harbor, brown bullhead (*Ictalurus nebulosus*) and white sucker (*Catostomus commersoni*), are affected by high prevalences of hepatic cholangiolar neoplasms (*43*). Although there are no studies documenting experimental induction in fish or rodents of the unique lesion type referred to as hydropic vacuolation of biliary epithelial cells/hepatocytes, this lesion type has been circumstantially (*27–30*) and statistically associated with contaminant exposure in winter flounder (*31,32*) and starry flounder (*34*). Therefore, certain hepatic lesions in fish, especially in comparison to lesions in other organ systems (*2*), are currently regarded as having utility as histological indicators or biological markers (biomarkers) of contaminant exposure and effects (*2,19–21,34,44*) and have become useful indicators of environmental degradation in marine ecosystems.

Accordingly, in the present study, we examined relationships between toxicopathic liver lesions and chemical contaminant levels in sediments, as well as in stomach contents, liver, and bile using various statistical approaches in three bottom-dwelling and bottom-feeding fish species (English sole, starry flounder, and white croaker) sampled annually between 1984 and 1988 as part of the Pacific Coast portion of the National Benthic Surveillance Project (NBSP) of the National Status and Trends Program of the National Oceanic and Atmospheric Administration. This multiyear study is the first to comprehensively examine the influences of various biological variables (fish age and gender) and contaminant-associated risk factors (e.g., site of capture and concentrations of selected chemical contaminants in sediment and fish) on the occurrence of hepatic lesions in fish species from a broad geographic range of marine and estuarine sites on the Pacific Coast.

Materials and Methods

Field Sampling

Fish and sediments were collected from 27 sites representing a broad gradient in types and concentrations of environmental chemical contaminants (*13*); pertinent data are presented in Table 1, and locations are

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Table 1. Pacific Coast sites at which English sole, starry flounder, and white croaker were collected and sampled in 1984–1988 as part of the National Benthic Surveillance Project[a]

Site category	Site	Site abbreviation	Location		Years sampled					Type of samples	Species sampled
			Latitude	Longitude	'84	'85	'86	'87	'88		
U	West Harbor Island, San Diego Bay, CA	WHIs	32°43.4′	117°12.4′				•	•	c	White croaker
U	North San Diego Bay, CA	nSDBy	32°43.2′	117°11.3′			•	•	•	c	White croaker
R	Dana Point, CA	DnPt	33°26.7′	117°41.5′	•	•	•			c	white croaker
HU	Cerritos Channel, CA	CrCh	33°45.0′	118°15.0′				•	•	c	White croaker
U	San Pedro Outer Harbor, CA	SPOHb	33°42.6′	118°15.4′		•	•	•	•	c	White croaker
U	Long Beach, CA	LnBh	33°44.6′	118°10.6′		•	•	•	•	c	White croaker
U	Seal Beach, CA	SlBh	33°44.1′	118°08.8′	•					c	White croaker
U	San Pedro Canyon, CA	SPCn	33°42.0′	118°15.7′	•					c	White croaker
U	West of Marina del Rey, Santa Monica Bay, CA	wSMBy	33°56.5′	118°33.3′			•		•	c	English sole
U	Monterey Bay, CA	MtBy	36°37.6′	121°52.3′		•	•	•		c	English sole
U	Moss Landing, CA	MsLg	36°48.1′	121°47.6′			•			p,b,s	English sole
U	San Pablo Bay, CA	SPbBy	38°02.9′	122°17.6′	•	•	•	•	•	c	White croaker, starry flounder
U	Castro Creek, CA	CsCk	37°58.9′	122°24.9′				•	•	c	White croaker, starry flounder
U	Southampton Shoal, CA	SoSh	37°53.2′	122°24.4′	•	•	•	•	•	c	White croaker, starry flounder
U	Oakland, CA	OakI	37°47.5′	122°20.3′	•					c	White croaker
U	Hunters Point, CA	HnPt	37°42.0′	122°21.5′	•	•	•	•	•	c	White croaker, starry flounder
HU	Oakland Estuary, CA	OkEs	37°47.0′	122°15.0′			•	•	•	c	White croaker
U	Redwood City, CA	RdCy	36°33.0′	122°11.0′			•	•	•	c	White croaker
R	Bodega Bay, CA	BdBy	38°18′.3	123°02.5′	•	•	•	•	•	c	English sole, white croaker starry flounder
U	Coos Bay, OR	CsBy	43°22.6′	124°12.5′	•	•	•		•	c	English sole, starry flounder
NU	Columbia River Estuary, OR	ClREs	46°13.2′	123°55.6′	•	•	•			c	Starry flounder
NU	Youngs Bay, OR	YnBy	46°10.2′	123°50.1′			•			p,s	Starry flounder
R	Nisqually Reach, WA	NsRh	47°06.7′	122°41.9′	•	•	•			c	English sole
U	Commencement Bay, WA	CmBy	47°16.7′	122°25.0′	•	•	•			c	English sole
U	Elliott Bay, WA	ElBy	47°35.5′	122°21.4′	•	•	•			c	English sole
NU	Boca de Quadra, AK	BdQd	55°16.5′	130°32.5′			•			c	English sole
NU	Chukchi Sea, Red Dog, AK	ChkS	70°	161°				•		c	Starry flounder

Abbreviations: c, complete set of samples; p, fish pathology; b, bile analysis; s, sediment metals; U, urban site; HU, highly urban site; NU, nonurban site; R, nonurban reference site.

[a]Only sites at which a total of at least 15 specimens of at least one species were captured and examined histopathologically are included.

shown in Figure 1. In general, sites were in a subtidal, sedimentary-depositional zone outside the zone of initial dilution of a point source for contaminants, or outside the zone of an authorized dumpsite, and in an area not subject to dredging, scouring, or slumping, as previously defined as criteria for site selection in the NBSP (*13*). Seventeen sites were located in or near urban embayments and fulfilled the NBSP criteria as zones of integration of multiple contaminant inputs; two other sites (Oakland Estuary, Cerritos Channel) were located in highly industrialized embayments impacted by adjacent multiple point sources of contaminants; the eight remaining sites were in nonurban embayments on the basis of minimal levels of sediment contaminants (*13*), of which three served as reference sites (Nisqually Reach, Washington; Bodega Bay, California, and Dana Point, California). Each site was represented by three stations, generally located <0.4 km apart. Stations for sediment collection were located along the trawl lines for fish collection. Standardized collection gear, sampling methods, and navigational equipment to determine the precise location of sampling stations (*13*) were used to maximize comparability of data over space and time

We collected fish by otter trawl using netting that was not chemically treated (*13*). Because toxicopathic liver lesions, including neoplasms, are more commonly found in older fish (*21,45*) fish of minimum size (≥15 cm, total length) were randomly selected from each haul and maintained (≤3 hr) in flowing fresh seawater until necropsy. We collected 30–60 fish per species for necropsy among the three stations at each site, depending on fish availability. Each fish was assigned a unique field identification number, weighed (total weight, g), measured (total length, mm), and killed by severing the spinal cord, followed by removal of otoliths for age determination. The abdominal cavity was opened, and the gall bladder, liver, gonad, and stomach contents were removed. A single tissue section ≤3 mm thick from the central portion along the longitudinal axis in grossly normal livers, with additional sections from regions containing gross lesions, and a transverse section of the gonad were collected in cassettes and preserved in Dietrich's fixative (*46*) for routine histological processing and examination. We collected bile from the gall bladder (10–12 fish/species/site) in amber-colored glass vials and placed approximately one-half of the remaining liver tissue (30 fish/species/site) in methylene chloride-rinsed glass vials and froze them at -80°C for determination of levels of fluorescent aromatic compounds (FACs) in bile, chlorinated hydrocarbons (e.g., PCBs), and chlorinated pesticides (e.g., DDTs). The remaining liver tissue from 30 fish/species/site was placed in a plastic, acid-rinsed vial and stored frozen (-80°C) until analyzed for selected trace elements (*13*). The total stomach contents from at least 10 fish/species/site were removed and composited in a methylene chloride-rinsed glass jar (up to three composites/species/site) and frozen (-80°C) for separate organic chemical and trace element analyses. We collected surficial sediments (top 2–3 cm) were collected using previously described protocols (*13,47*).

Laboratory Analyses

Tissues preserved in the field were routinely processed for paraffin embedding, sectioned at a 4–5-μm thickness, and stained with Mayer's or Gill's hematoxylin and alcoholic eosin-Y (*48*). Histologic slides were examined by a system that ensured histopathologists were unaware of the site of origin for each specimen. Lesion classification followed widely accepted criteria (*18,49*). For reporting and statistical analyses, we placed hepatic lesions into the following diagnostic categories: 1) neoplasms

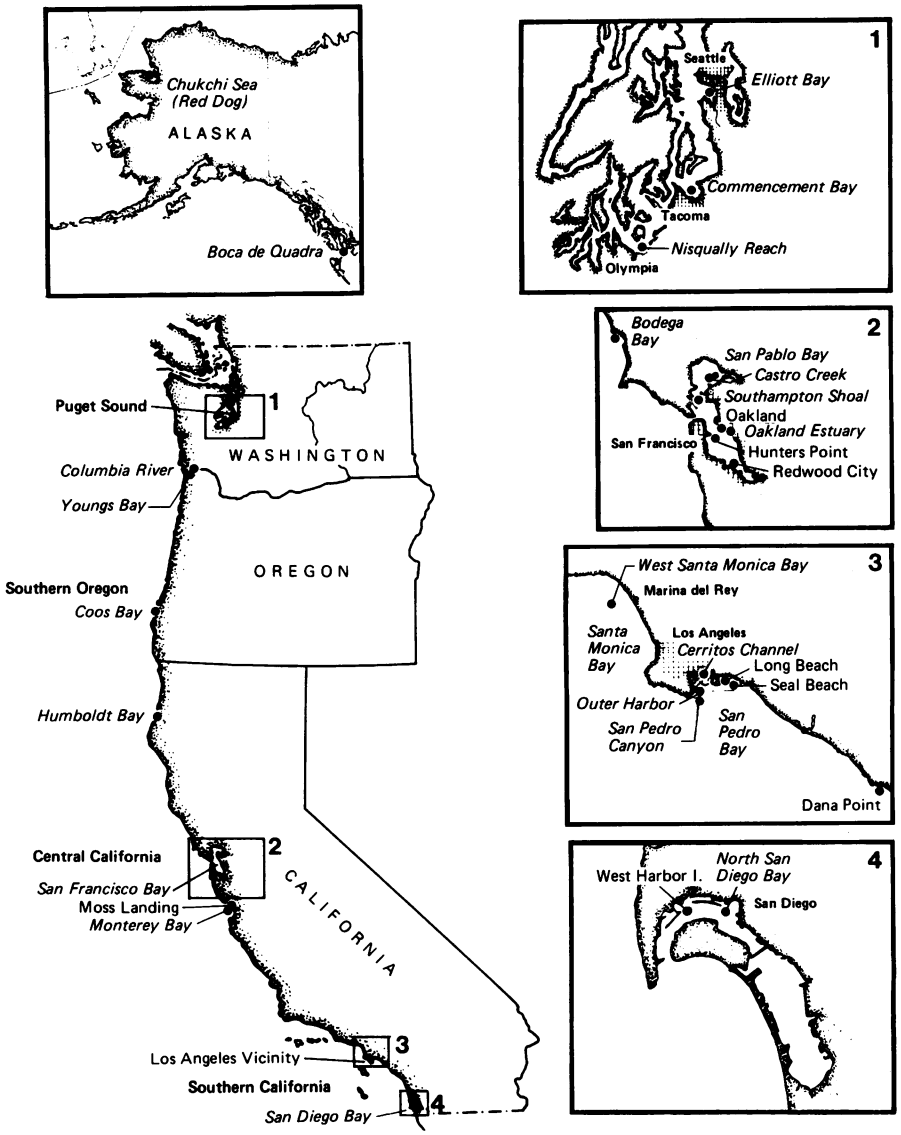

Figure 1. Map of the Pacific Coast of the contiguous United States showing locations of sampling sites.

(hepatocellular adenoma, hepatocellular carcinoma, cholangioma, cholangiocellular carcinoma, and mixed hepatobiliary carcinoma); 2) putatively preneoplastic foci of cellular alteration (FCA) (eosinophilic focus, clear cell focus, basophilic focus); 3) nonneoplastic proliferative lesions (Prolif) (hepatocellular regeneration, biliary hyperplasia or proliferation, presumptive oval cell proliferation, cholangiofibrosis, and increased mitotic activity in hepatocellular/biliary epithelial cells); 4) specific or unique degenerative/necrotic conditions (SDN) (megalocytic hepatosis, hepatocellular nuclear pleomorphism, and, rarely, spongiosis hepatis); 5) nonspecific necrotic lesions not associated with visible infectious agents (necrosis) (hepatocellular or biliary coagulative necrosis, hyalinization, pyknosis, and karyorrhexis); and 6) hydropic vacuolation of biliary epithelial cells or hepatocytes (HydVac). The microscopic anatomy of lesions in categories 1–5 is documented in English sole (*15–18*). Hydropic vacuolation is a unique lesion visualized by light microscopy as a severe hydropic ballooning of the cytoplasmic space, and by transmission electron microscopy as massive distension of the perinuclear cisternae of the rough endoplasmic reticulum (*27–29,49–51*). All cases of hydropic vacuolation in this study showed a diffuse distribution of affected cells; in contrast to observations in winter flounder (*27-29,49–51*), focal or nodular lesions consisting of hydropically vacuolated cells were rarely detected and only in white croaker.

We determined fish age for a subset of the total number of specimens in each target species. This subset consisted, at a minimum, of the entire sample for a particular species collected in at least one cycle within a geographic area (e.g., San Francisco Bay, Puget Sound, southern California). Age was determined by counting the number of clearly defined opaque zones of whole otoliths (*53*), or, in the case of white croaker otoliths, which cannot be reliably aged by this method, by a specialized technique involving embedding and sectioning (*54*). For the remaining fish, age was estimated from length using gender-specific age-length curves constructed for each species from particular geographic areas (*40*). Sample sizes for these age-length curves are given in an NBSP document (*40*) and generally ranged from 30 to 120. When gender data were not available or where insufficient numbers of a gender prevented meaningful age-length regression, age was estimated from age-length equations for combined genders.

We analyzed sediments and stomach contents for a broad spectrum of PAHs, chlorinated hydrocarbons (CHs), and selected trace elements (*13,47,55–57*). Liver tissues were analyzed for selected CHs and selected trace elements, but not for PAHs due to the extensive metabolism of these labile compounds to more polar biliary metabolic products (*58*). Therefore, FACs in bile were measured using the procedures of Krahn et al. (*59,60*) to estimate PAH exposure in fish.

Although levels of many individual PAHs (n = 23), CHs (n = 21), and trace elements (n = 15) were determined in sediments, stomach contents, and liver tissue (CHs and trace elements only) (*56,57*), levels of chemical groups were also computed for reporting purposes and statistical analyses. Concentrations of PAHs and CHs in sediments and stomach contents and CHs in liver were grouped into the following categories: low molecular weight PAHs containing two to three benzene rings (ΣLAHs), high molecular weight PAHs containing four to six benzene rings (ΣHAHs), polychlorinated biphenyls (ΣPCBs, including tri- to decachlorobiphenyls), ΣDDTs (DDT and its derivatives, DDD and DDE), chlordanes (α-chlordane and *trans*-nonachlor), dieldrin, and hexachlorobenzene. Specific compounds and concentrations in each of these classes are documented elsewhere (*40,56*). For biliary FACs, the data are reported (*56*) as total levels of compounds fluorescing at wavelength pairs appropriate for metabolites of benzo[*a*]pyrene (FACs-H) and naphthalene (FACs-L), which are common higher and lower molecular weight aromatic compounds, respectively, in urban sediments (*60*). For the essential or toxic elements in sediments, stomach contents, and liver, two groups were formed on the basis of their pattern of strong covariance in sediments (*13*) as shown by principal components analysis (*61*): metals 1 consisted of the summed individual concentrations of copper, zinc, lead, and tin, and metals 2 was composed of summed individual levels of nickel, chromium, and selenium.

Statistical Analyses

To determine if significant differences in lesion prevalences in a particular species existed between the reference site and the individual test sites, we computed the G-statistic (*62*) using the lesion prevalence at the closest reference site as the expected value (Table 1). Analyses were performed on site-specific lesion prevalence data for the five years combined, and the critical level of significance was set at $p \leq 0.05$.

We used stepwise logistic regression (*63*) to identify statistically significant relationships between potential risk factors and lesion occurrence. This epidemiological method is commonly used on binomial or proportional data to examine the influence of multiple risk factors on the probability of disease occurrence as well as exposure–response relationships and allows for simultaneous adjustment for risk factors included in the regression by iterative model fitting. This method has been successfully applied to the epizootiology of hepatic and/or renal lesions in English sole (*19–21*), rock sole and starry flounder (*34*), winter flounder (*31,32*), common dab (*36*), and European flounder (*37*).

Contaminant classes evaluated as risk factors for hepatic lesions were: ∑HAHs and ∑LAHs in sediments and stomach contents; ∑PCBs, ∑DDTs, hexachlorobenzene, chlordanes, dieldrin, and metals 1 and metals 2 in sediments, stomach contents, and liver tissue; and FACs-L and FACs-H in bile. Although specific PCB congeners were included in the ∑PCBs class, unique congeners were not assessed as risk factors. These chemical classes were selected for analyses relating contaminant exposure to disease risk because they are common indices of urban-associated anthropogenic pollution (*13,25*) and represent broad classes of chemicals with documented toxic or carcinogenic potential in vertebrates (*64*), including fish (*1*).

Logistic models were fitted using the PECAN module of the EGRET statistical package, version III (Statistics and Epidemiology Research Corporation, Seattle, Washington). Risk factors were considered significant at $p \leq 0.05$, with independent analyses performed for all lesion categories. We performed two types of analyses. The first determined the calculated odds ratio as an estimate of relative risk for lesions in individual fish in relation to the variables of site, gender, and age or estimated age. In this analysis the odds ratio represents the degree of association between a risk factor and lesion occurrence (*65*) as determined from variable coefficients of the logistic regression equations (*66,67*). We calculated odds ratios for lesion categories at a site and interpreted them relative to the lesion occurrence at the reference site(s) for each species. Increased probabilities of lesion occurrence were indicated by odds ratios greater than 1.000. Odds ratios for age were interpreted for each additional year in age. Because separate chemical analyses for tissues from individual fish were not performed, risk factors of actual chemical exposure could not be assessed in this analysis.

The second analysis determined the significance of the relationships between prevalences of lesions at the sampling sites and discrete risk factors, such as levels of contaminants in sediments and fish tissues, while adjusting for mean fish age and gender ratio. Each year's data for a species at a site were treated as an independent occurrence. We performed separate analyses for each contaminant class (risk factor) discussed above, with results expressed as the proportion of variation in lesion prevalence attributable to significant risk factors. As an adjunct analysis, the significance ($p<0.05$) of correlation coefficients for concentrations of different chemical classes within the same compartment (e.g., sediment) and concentrations of the same chemical class among the compartments (e.g., PCBs in sediment and liver) was computed (*62*). These correlative data are reported in full elsewhere (*40*). Because concentrations of risk factors assessed in this analysis were often significantly correlated or covariant (*40,56*), it was not mathematically possible to include all risk factors into one multivariate analysis. Consequently, we evaluated relationships between concentrations of chemical classes and lesion prevalences at the sites separately, adjusting for age and gender.

Results

Chemical Characterization of Sediments and Fish Tissues

Maximal mean concentrations of most organic contaminants and essential and toxic elements in sediment and fish tissues were present in the highly urbanized areas, and many CHs were extensively bioaccumulated in liver. However, no correlations were found between concentrations of most of the trace elements measured in sediment and liver (*56,57*). The highest levels of organic contaminants were found at urban sites in Puget Sound, San Francisco Bay, the Los Angeles vicinity, and San Diego Bay. Although it is not possible to present the entire chemistry data set here, examples of maximal mean concentrations in sediments, liver, and bile are

Table 2. Maximal mean concentrations of selected chemical contaminants in sediments and fish tissues collected at selected sites sampled on the Pacific Coast in the National Benthic Surveillance Project (1984–1988)[a]

	Geographic areas (sites)			
Chemicals	Puget Sound	San Francisco Bay	Los Angeles area	San Diego Bay
Sediments				
∑LAHs	1,300 (ElBy)	1,600 (HnPt)	340 (wSMBy)	340 (nSDBy)
∑HAHs	4,600 (ElBy)	8,700 (HnPt)	1,300 (SPOHb)	2,800 (nSDBy)
∑PCBs	500 (ElBy)	260 (OkEs)	410 (SPOHb)	430 (nSDBy)
∑DDTs	17 (ElBy)	470 (SoSh)	670 (SPOHb)	70 (WHIs)
Chlordanes	1 (CmBy)	6 (OkEs)	15 (LnBh)	3 (nSDBy)
Dieldrin	2 (ElBy)	3 (OkEs)	1 (LnBh)	0.1 (nSDBy)
Hexachlorobenzene	5 (CmBy)	1 (HnPt)	1 (SPOHb)	0.1 (nSDBy)
Metals 1	420 (ElBy)	240 (HnPt)	400 (LnBh)	410 (nSDBy)
Metals 2	150 (ElBy)	730 (SPbBy)	140 (SlBh)	60 (nSDBy)
Liver tissue				
∑PCBs	11,000 (ElBy, ES)	7,000 (HnPt, SF) 6,800 (OkEs, WC)	15,000 (SlBh, WC)	8,400 (WHIs, WC)
∑DDTs	1,100 (ElBy, ES)	1,900 (SPbBy, SF) 1,800 (HnPt, WC)	26,000 (SPOHb, WC)	1,400 (nSDBy, WC)
Chlordanes	250 (CmBy, ES)	330 (HnPt, SF) 260 (OkEs, WC)	1,700 (LnBh, WC)	190 (nSDBy, WC)
Dieldrin	34 (CmBy, ES)	300 (HnPt, SF) 170 (RdCy, WC)	190 (LnBh, WC)	50 (nSDBy, WC)
Hexachlorobenzene	150 (CmBy, ES)	12 (HnPt, SF) 8 (OkEs, WC)	9 (LnBh, WC)	4 (nSDBy, WC)
Biliary FACs				
FACs-H	1,000 (ElBy, ES)	390 (SoSh, SF) 760 (OkEs, WC)	2,900 (CrCh, WC)	2,300 (WHIs, WC)
FACs-L	150,000 (ElBy, ES)	160,000 (SPbBy, SF) 140,000 (OkEs, WC)	220,000 (CrCh, WC)	133,000 (nSDBy)

Abbreviations: LAHs, low molecular weight polycyclic aromatic hydrocarbons; HAHs, high molecular weight polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls, FACs-H, aromatic compounds fluorescing at benzo[a]pyrene wavelengths; FACs-L, aromatic compounds fluorescing at naphthalene wavelengths; ES, English sole; SF, starry flounder; WC, white croaker.
[a]Concentrations shown are in ng/g (dry weight), except for biliary FACs concentrations (ng/g, wet weight). See Table 1 for site abbreviations.

presented in Table 2. Further information on levels of specific chemicals measured in sediments, stomach contents, liver, and bile used in the logistic analyses below are in previously published reports (*13,14,24, 25,47,56,57,68*).

Lesion Occurrence

English sole. Among the northern sites (Fig. 2A), prevalences of neoplasms, Prolif, and SDN were higher at both the Commencement Bay and Elliott Bay sites in Puget Sound than at the reference site. Elliott Bay sole also had higher prevalences of FCA and necrotic lesions. HydVac did not occur. Prevalences of neoplasms and necrotic lesions were <10%, with higher prevalences of FCA, Prolif, and SDN in sole from these two urban sites. No lesions were detected in sole at the Boca de Quadra site, and Coos Bay sole were affected only with necrotic lesions. For the southern sites (Fig. 2B), prevalences of all lesions were low (<10%) at all sites, except for the Monterey Bay site where higher prevalences of Prolif, SDN, and necrosis were detected.

Substantially elevated relative risks of neoplasms, FCA, Prolif, and SDN were calculated for sole from Elliott Bay (Table 3). For example, a sole from Elliott Bay was >34 times more likely to be affected by a neoplasm than a fish of comparable age and gender from the reference sites. Residence at Monterey Bay was associated with an increased risk of necrosis. Age was a risk factor only for neoplasms, and males were more likely to be affected by necrotic lesions. Age ranges and means ± SD (in years) of sole affected with lesions were: neoplasms (5–9, 6.1 ± 1.5); FCA (3–9, 5.3 ± 1.8); Prolif (3–10, 4.8 ± 1.7); SDN (1–10, 4.6 ± 1.7); necrosis (1–9, 4.4 ± 1.9).

Starry flounder. Prevalences of lesions other than HydVac were generally below 10%, and neoplasms were not detected (Fig. 3). Significant intersite differences in prevalences other than for HydVac were shown only for FCA and SDN in flounder from Hunters Point in San Francisco Bay. HydVac was detected at eight of the nine sites; however, higher prevalences were shown only at three urban sites within San Francisco Bay.

For FCA there was an increased risk, relative to the reference site at Bodega Bay, in flounder from the urban sites of Castro Creek and Hunters Point in San Francisco Bay, whereas the relative risk for SDN was higher only at Hunters Point (Table 3). Several other sites were associated with an increased risk of HydVac, and, with the exception of Youngs Bay, all were urban sites within San Francisco Bay. The highest relative risks, especially for HydVac, were generally shown at the urban sites of Castro Creek and Hunters Point. Age was a risk factor for FCA, Prolif, and HydVac. Age ranges and means ± SD (in years) of flounder affected with lesions were: FCA (5–14, 9.5 ± 3.5); Prolif (4 –12, 8.3 ± 1.9); SDN (2–12, 4.8 ± 2.3); necrosis (2–9, 5.3 ± 2.3); HydVac (2–16, 6.4 ± 3.0). Gender was not a risk factor for any lesion.

White croaker. All lesion categories were detected in croaker from the northern (Fig. 4A) and southern (Fig. 4B) sampling sites; HydVac was most frequently detected, at prevalences ranging from <1% to nearly 40%. At the Bodega Bay reference site (northern sites), FCA and necrosis were not detected, and prevalences of all lesions were very low. The only neoplasms (cholangiocellular carcinomas) diagnosed among the northern sites were in croaker from Bodega Bay; both affected fish were at least 11 years old (*13*). Several lesion types occurred at higher prevalences at urban sites: FCA at Hunters Point and Redwood City; Prolif and SDN at the Oakland Estuary; and HydVac and necrosis at all San Francisco Bay sites except Redwood City. At the southern sites, lesion prevalences other than SDN and HydVac were <15%. At the southern reference site of Dana Point, prevalences of SDN were low, HydVac prevalence was about 10%, and neoplasms, FCA, Prolif, and necrosis were not detected. Neoplasm prevalences were higher in croaker from San Pedro Outer Harbor, Cerritos Channel, and Long Beach; FCA was higher only at the latter two sites. Prevalences of Prolif were higher at the west Harbor Island site in San Diego Bay, Cerritos Channel, and the Outer Harbor and Seal Beach sites in San Pedro Bay; higher prevalences of SDN were detected at Cerritos Channel and San Pedro Outer Harbor. Southern sites with HydVac prevalences higher than those at the reference site were Cerritos Channel, San Pedro Outer Harbor, San Pedro Canyon, and Long Beach. Prevalences of necrosis were higher at four of the eight southern sampling sites. Southern sites with the most lesion categories with higher prevalences were Cerritos Channel (all lesions) and San Pedro Outer Harbor (five lesions). Among sites in San Diego Bay, croaker from the west Harbor Island site were most frequently affected, whereas prevalences of HydVac at San Diego sites were lower than that at the reference site.

Except for SDN and necrosis, all lesion types showed a higher risk of occurrence with increasing age (Table 3). Age ranges and means ± SD (in years) of affected croaker were: neoplasms (5–24, 10.4 ± 6.3); FCA (5–16, 10.5 ± 3.7); Prolif (1–24, 7.9 ± 6.3); SDN (1– 10, 4.6 ± 2.9); necrosis (0–16, 4.4 ± 4.1); HydVac (1– 23, 8.1 ± 4.0). Being male was a risk factor only for neoplasms. Urban sites in the Los Angeles vicinity, San Diego Bay, and San Francisco Bay were associated with increased risk of lesion occurrence (Table 3). Sites and lesions showing increased risks were: San Pedro Outer Harbor (neoplasms, Prolif, SDN, necrosis, HydVac); Cerritos Channel

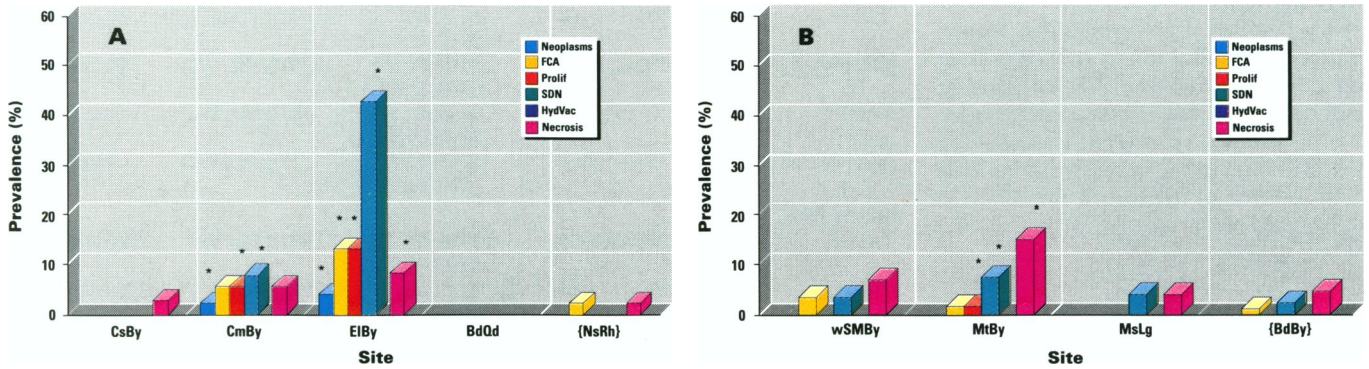

Figure 2. Prevalences of toxicopathic liver lesions in English sole captured at northern (A) and southern (B) sites in the National Benthic Surveillance Project (1984–88). FCA, foci of cellular alteration; Prolif, nonneoplastic proliferative lesions; SDN, specific degenerative/necrotic lesions; HydVac, hydropic vacuolation; Necrosis, nonspecific necrotic lesions. The number of fish examined per site is given below each site abbreviation (see Table 1 for site definitions). The reference site is shown at the far right of the graph and is indicated by braces. (*) Lesion prevalences significantly higher than those detected at the reference site ($p \leq 0.05$).

Table 3. Calculated odds ratios/relative risks for significant ($p < 0.05$) risk factors (site of capture, age, and gender) for six categories of hepatic lesions in English sole (ES), starry flounder (SF), and white croaker (WC)[a]

Lesion	Species	Risk factor	Odds ratio/relative risk
Neoplasms	English sole	Elliott Bay	34.190
GM = 0.139E-3, ES		Age	1.750
= LND, SF	White croaker	San Pedro Outer Harbor	12.390
= 0.793E-3, WC		Age	1.442
		Male	31.447
Foci of cellular alteration	English sole	Elliott Bay	12.430
GM = 0.185E-2, ES	Starry flounder	Castro Creek	12.640
= 0.423E-3, SF		Hunters Point	6.784
= 0.288E-3, WC		Age	1.396
	White croaker	Cerritos Channel	15.340
		Age	1.333
Proliferative lesions	English sole	Elliott Bay	18.790
GM = 0.915E-2, ES	Starry flounder	Age	1.257
= 0.285E-2, SF	White croaker	Cerritos Channel	88.590
= 0.297E-1, WC		San Pedro Outer Harbor	26.740
		Oakland Estuary	112.000
		Age	1.380
Specific degeneration/necrosis	English sole	Elliott Bay	24.120
GM = 0.344, ES	Starry flounder	Hunters Point	9.326
= 0.105E-1, SF	White croaker	Cerritos Channel	30.210
= 0.171E-1, WC		San Pedro Outer Harbor	11.810
		Oakland Estuary	7.301
		Long Beach	3.606
Hydropic vacuolation	Starry flounder	Southampton Shoal	5.925
GM = LND, ES		Hunters Point	15.630
= 0.180E-1, SF		Castro Creek	6.037
= 0.324E-1, WC		Youngs Bay	6.297
		Age	1.271
	White croaker	Castro Creek	7.073
		Oakland Estuary	7.972
		San Pablo Bay	7.454
		Southampton Shoal	3.495
		Oakland	11.950
		Hunters Point	5.229
		Redwood City	7.292
		Long Beach	22.490
		San Pedro Outer Harbor	12.490
		Seal Beach	15.650
		Cerritos Channel	13.510
		San Pedro Canyon	6.430
		West Harbor Island	4.523
		Age	1.277
Necrosis	English sole	Monterey Bay	4.400
GM = 0.659E-1, ES		Male	2.688
= 0.228E-1, SF	Starry flounder	None	
= 0.643E-2, WC	White croaker	Cerritos Channel	15.550
		San Pedro Outer Harbor	9.238
		San Pedro Canyon	19.440
		Oakland Estuary	21.650
		Hunters Point	5.437

[a]Odds ratios for the site of capture are interpreted relative to the combined data for the reference sites, except for hydropic vacuolation in croaker where the reference site was Bodega Bay. Odds ratios for age (in years) represent the effect of each additional year of age on the odds of disease occurrence. GM, grand mean; $N = 413$ (ES), 718 (SF), 1333 (WC); LND, lesion not detected.

(FCA, Prolif, SDN, necrosis, HydVac); Long Beach (SDN, HydVac); San Pedro Canyon (HydVac, necrosis); Oakland Estuary (Prolif, SDN, HydVac, necrosis); and Hunters Point (HydVac, necrosis). An increased relative risk for HydVac was determined at the following additional sites, as compared to the prevalence at Bodega Bay: in San Francisco Bay, Castro Creek, San Pablo Bay, Southampton Shoal, Oakland, and Redwood City; in the Los Angeles vicinity, Seal Beach; and in San Diego Bay, West Harbor Island.

Lesion Prevalences and Chemical Risk Factors

The results of logistic analyses testing the significance of chemical parameters in sediments (potential exposure), stomach contents (dietary uptake), liver tissue (bioaccumulation), and bile (estimate of recent exposure to and metabolism of PAHs) as risk factors for hepatic lesions are presented in Tables 4–6.

English sole. Of chemicals in sediment, the ΣLAHs, ΣHAHs, ΣPCBs, and metals 1 were risk factors for all lesions in sole except necrosis, which showed no risk factors (Table 4). Separate risk factors accounted for between 38 and 68% of the intersite variation in prevalence. ΣDDTs and chlordanes were risk factors for SDN and Prolif, but explained less variation than the above factors. Dieldrin was also a risk factor for neoplasms and Prolif. Concentrations of chemical classes in sediment, especially the ΣLAHs, ΣHAHs, ΣPCBs, and metals 1, were significantly covariant (*40,56*), with correlation coefficients (r) ranging from 0.841 to 0.997. Other covariant sediment measures were: ΣDDTs versus ΣPCBs ($r = 0.430$) and chlordanes ($r = 0.773$); dieldrin versus ΣLAHs ($r = 0.473$), ΣHAHs ($r = 0.549$), and metals 1 ($r = 0.576$); and chlordanes versus metals 1 ($r = 0.576$).

In stomach contents, ΣLAHs, ΣHAHs, ΣDDTs and ΣPCBs were risk factors for FCA, SDN, and Prolif. The only risk factor for neoplasms was chlordanes, which was also a risk factor for SDN and Prolif. Metals 1 was a risk factor for FCA, SDN, and Prolif. Chemical concentrations in stomach contents were also covariant ($0.692 \leq r \leq 0.999$), excepting PAHs and chlordanes, chlordanes and metals 1, and ΣPCBs and metals 1 (*40*).

Biliary FACs-L or -H were risk factors for all lesions except necrosis. Hepatic concentrations of ΣDDTs and ΣPCBs were risk factors for neoplasms, FCA, SDN, and Prolif; ΣDDTs was a risk factor for necrosis. Levels of some risk factors in bile and liver were covariant; FACs-L versus ΣDDTs ($r = 0.651$) and ΣPCBs ($r = 0.819$), and ΣDDTs versus ΣPCBs ($r = 0.656$).

Starry flounder. Several chemical classes were risk factors for the lesions FCA, SDN, and HydVac in flounder (Table 5). In sediments, ΣLAHs, ΣHAHs, ΣPCBs, and metals 1 were risk factors for SDN and HydVac; hexachlorobenzene was also a risk factor for HydVac. The ΣLAHs, ΣHAHs, and ΣPCBs explained simlar proportions of the prevalence variation for SDN, and their sediment concentrations covaried ($0.362 \leq r \leq 0.999$) (*40*). Moreover, metals 1 concentrations in sediment covaried with ΣLAHs ($r = 0.557$), ΣHAHs ($r = 0.657$), ΣPCBs ($r = 0.811$), and hexachlorobenzene ($r = 0.777$). For HydVac, similar proportions of the prevalence variation were accounted for by ΣLAHs, ΣHAHs, ΣPCBs, hexachlorobenzene, and metals 1, the concentrations of which were covariant ($0.362 \leq r \leq 0.999$); hexachlorobenzene most strongly covaried with ΣPCBs ($r = 0.709$) and metals 1 ($r = 0.777$).

In stomach contents, several risk factors were identified for FCA and HydVac. The covariant measures of ΣDDTs and 1 and ΣPCBs ($r = 0.845$) were risk factors for FCA, whereas ΣHAHs, ΣDDTs, and

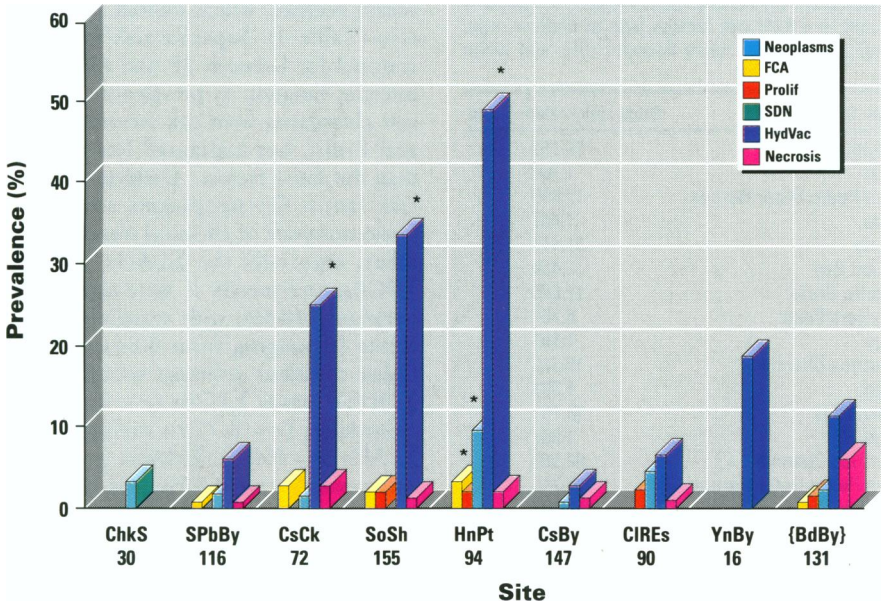

Figure 3. Prevalences of toxicopathic liver lesions in starry flounder at sites sampled in the National Benthic Surveillance Project (1984–88). FCA, foci of cellular alteration; Prolif, nonneoplastic proliferative lesions; SDN, specific degenerative/necrotic lesions; HydVac, hydropic vacuolation; Necrosis, nonspecific necrotic lesions. The number of fish examined per site is given below each site abbreviation (see Table 1 for site definitions). The reference site is shown at the far right of the graph and is indicated by braces. (*) Lesion prevalences significantly higher than those detected at the reference site ($p \leq 0.05$).

ΣPCBs were risk factors for HydVac; concentrations of the CH groups were covariant ($0.669 \leq r \leq 0.857$). None of the CHs were correlated with PAHs in stomach contents (*40*).

Chemical risk factors identified in liver and bile were similar to those in sediments and were mainly shown for SDN and HydVac. The covariant ΣPCBs, chlordanes, and dieldrin ($0.713 \leq r \leq 0.868$) were risk factors for SDN. Metals 1 in liver was a risk factor for SDN, reflecting its lack of correlation with any CHs or PAHs (*40*). PAH exposure as reflected by the correlated measures of FACs-H and -L ($r = 0.814$) was a risk factor for HydVac, as were the covariant measures of liver ΣDDTs, ΣPCBs, chlordanes, and dieldrin ($0.691 \leq r \leq 0.868$).

White croaker. At least one chemical class in sediment was a risk factor for all lesions in croaker, excepting neoplasms (Table 6). ΣLAHs and ΣHAHs were risk factors for FCA, while ΣDDTs, ΣPCBs, chlordanes, and metals 1 were risk factors for SDN and HydVac. Dieldrin and hexachlorobenzene were also risk factors for HydVac; ΣDDTs and ΣPCBs were risk factors for Prolif. Risk factors for necrosis were ΣLAHs, ΣHAHs, and ΣPCBs. PAH measures in sediment were covariant ($r = 0.967$), as were metals 1 and ΣHAHs ($r = 0.544$). PAH measures also covaried with ΣPCBs and hexachlorobenzene ($0.362 \leq r \leq 0.422$), as did ΣPCBs with metals 1 ($r = 0.839$). Chlordanes covaried with metals 1 ($r = 0.595$) and ΣPCBs ($r = 0.514$), which in turn covaried with ΣDDTs ($r = 0.445$). Dieldrin levels covaried with all chemical classes in sediment ($0.306 \leq r \leq 0.700$) except ΣDDTs. Overall, PAHs in sediments were associated with FCA and necrosis, whereas the groups within the CHs and metals 1 were risk factors for SDN, HydVac, Prolif, and necrosis.

Chemical risk factors in stomach contents, primarily CHs, were associated with SDN, HydVac, and Prolif. Risk factors for SDN were ΣDDTs, ΣPCBs, chlordanes, and metals 1; risk factors for HydVac were ΣDDTs, ΣPCBs, and chlordanes. Concentrations of the CHs were covariant ($0.624 \leq r \leq 0.727$). The correlated measures of ΣDDTs and ΣPCBs ($r = 0.688$) were risk factors for Prolif; hexachlorobenzene appeared to be independent because it was not covariant with ΣDDTs or ΣPCBs (*40*). Hexachlorobenzene was the only risk factor for necrosis.

Levels of biliary FACs-L or -H were associated with FCA, SDN, HydVac, Prolif, and necrosis. Risk factors in liver included ΣDDTs for SDN, HydVac, Prolif, and necrosis; ΣPCBs for SDN and HydVac; chlordanes and dieldrin for HydVac; metals 1 for SDN and Prolif; and metals 2 for SDN and HydVac. ΣDDTs as a risk factor for SDN, HydVac, and Prolif paralleled results for sediments, reflecting the correlation between sediment and liver ΣDDTs ($r = 0.772$). Metals 1 and 2 were risk factors for SDN, HydVac, and Prolif, but their levels strongly covaried with ΣDDTs ($0.556 \leq r \leq 0.608$), a highly significant risk factor for all three lesions.

Discussion

Biological Risk Factors for Hepatic Lesions

Age. The risk of occurrence of several lesions increased with age, consistent with other studies in bottomfish, including

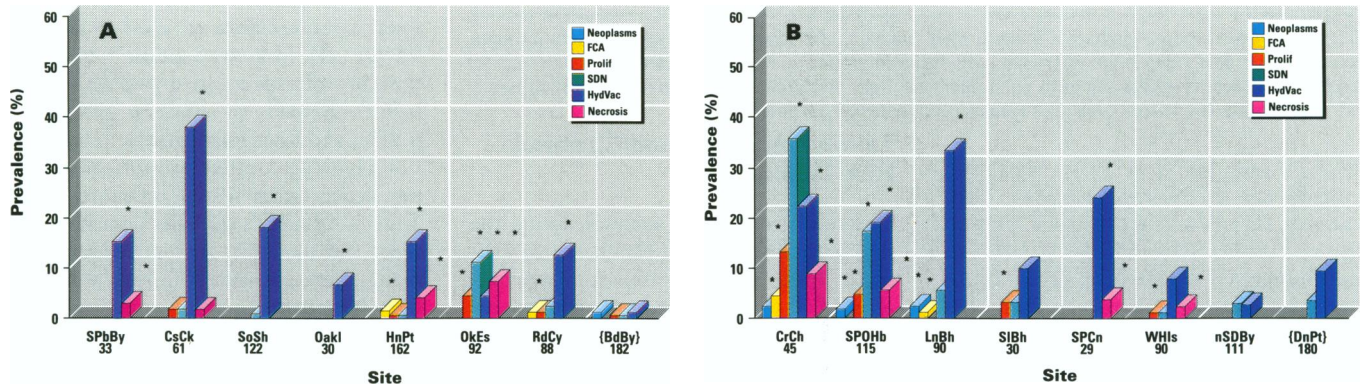

Figure 4. Prevalences of toxicopathic liver lesions in white croaker captured at northern (A) and southern (B) sites in the National Benthic Surveillance Project (1984–88). FCA, foci of cellular alteration; Prolif, nonneoplastic proliferative lesions; SDN, specific degenerative/necrotic lesions; HydVac, hydropic vacuolation; Necrosis, nonspecific necrotic lesions. The number of fish examined per site is given below each site abbreviation (see Table 1 for site definitions). The reference site is shown at the far right of the graph and is indicated by braces. (*) Lesion prevalences significantly higher than those detected at the reference site ($p \leq 0.05$).

Table 4. Chemical compounds or classes of compounds in sediment, stomach contents, liver, and bile showing significant positive associations by logistic regression ($p < 0.05$) with site-specific prevalences of selected categories of idiopathic liver lesions in English sole[a]

Chemical class/compartment	*n*	*p* - value/% total variance				
		Neoplasms	FCA	SDN	Prolif	Necrosis
ΣLAHs						
Sediment	22	0.020/54	<0.001/52	<0.001/45	<0.001/68	ns
Stomach contents	9	ns	<0.001/65	<0.001/75	<0.001/73	ns
Bile (FACs-L)	20	0.032/15	<0.001/26	<0.001/35	0.002/21	ns
ΣHAHS						
Sediment	22	0.004/48	<0.001/40	<0.001/38	0.003/55	ns
Stomach contents	9	ns	<0.001/65	<0.001/75	<0.001/73	ns
Bile (FACs-H)	20	ns	0.01/13	<0.001/22	<0.001/22	ns
ΣDDTs						
Sediment	22	ns	ns	<0.001/17	0.007/12	ns
Stomach contents	9	ns	0.003/49	<0.001/74	<0.001/82	ns
Liver	13	0.003/49	0.001/34	<0.001/28	0.001/29	0.037/27
ΣPCBs						
Sediment	22	0.004/53	<0.001/49	<0.001/45	<0.001/55	ns
Stomach contents	9	ns	0.004/29	<0.001/46	0.00570	ns
Liver	13	0.003/64	<0.001/60	<0.001/24	0.001/66	ns
Chlordanes						
Sediment	22	ns	ns	<0.001/18	0.005/15	ns
Stomach contents	9	0.007/70	ns	0.002/23	0.006/40	ns
Dieldrin						
Sediment	22	0.017/35	ns	ns	0.001/20	ns
Metals 1						
Sediment	20	0.003/57	<0.001/46	<0.001/65	<0.001/57	ns
Stomach contents	6	ns	0.003/72	0.001/74	0.002/78	ns

Abbreviations: FCA, foci of cellular alteration; SDN, specific degenerative/necrotic lesions; Prolif, proliferative lesions; *n*, number of sites; ns, not significant; LAHs, low molecular weight polycyclic aromatic hydrocarbons; HAHs, high molecular weight polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls, FACs-H, aromatic compounds fluorescing at benzo[a]pyrene wavelengths; FACs-L, aromatic compounds fluorescing at naphthalene wavelenghts; ES, English sole; SF, starry flounder; WC, white croaker.

[a]Analyses were performed separately while adjusting for mean age and gender ratio (female:male). Table indicates *p*-value, and percent of total variance in lesion prevalence explained by risk factor (reduction in scaled deviance).

walleye (*Stizostedion vitreum*) and brown bullhead (*Ameiurus nebulosus*) (*45,69,70*), winter flounder (*27,28,31,32*), English sole (*19–21*), ruffe (*Gymnocephalus cernua*) (*71*), bream (*Abramis brama*) (*72*), and European flounder (*35,37*). Specifically, the relative risk of neoplasm occurrence in sole and croaker increased directly with age, as in early studies on English sole in Puget Sound (*21*). The age of earliest occurrence of neoplasms in both species (5 years) was higher than that reported in studies in Puget Sound English sole (*21*), and was probably a function of targeting larger and older fish; the mean age of tumor-bearing sole (~6 years) in both studies was similar. The far higher mean age of tumor-bearing croaker (~10 years) suggests a longer latency period in the development of liver neoplasms, or a species-related resistance to the effects of exposure to hepatocarcinogenic chemicals as compared to English sole, or both.

Preneoplastic FCA and nonneoplastic proliferative lesions were more likely to occur in older flounder and croaker, as shown in English sole (*21*). The age of earliest occurrence (5 years) and mean age in flounder and croaker affected with FCA (~10 years) were also far higher than in previous studies in English sole (0+, 5 years, respectively) (*21*), again suggesting a longer latency or a relative resistance in these species to the development of precursor lesions in hepatic neoplasia. This possibility is strongly supported in flounder by the fact that no neoplasms and low FCA prevalences were detected in this study, consistent with findings in Puget Sound field studies (*33,34,73*), as well as the constitutively lower activation and increased detoxication of PAHs demonstrated in flounder relative to English sole (*74*). In contrast to a previous study (*21*), the present results indicated age was not a risk factor for either FCA or nonneoplastic proliferative lesions in sole. Likely reasons that these lesions were not linked to advancing age in this study are the lower sample size (413 versus 1083) and the lower lesion prevalences detected as a result of selection of a higher proportion of sampling sites categorized as relatively uncontaminated. In the study by Rhodes et al. (*21*), seven of the eight sampling sites were located in highly urban areas of Puget Sound with sediments containing high levels of anthropogenic contaminants (*15*), and hepatic lesion prevalences were consequently far higher than those detected here.

Age was not a risk factor for SDN in any species, consistent with previous findings in English sole (*21*). Moreover, the age of earliest occurrence of SDN in all three species (1–2 years) was similar to earlier data in English sole (*21*) and supports the view that lesions in this category are inducible over a shorter time frame than neoplasms, FCA, or proliferative lesions and may represent the earlier-occurring cytotoxic effects of exposure to hepatotoxicants and carcinogens (*18*). The risk of HydVac was associated with increasing age in both flounder and croaker, as in winter flounder (*28,31,32*) and rock sole (*Pleuronectes bilineata*) (*34*). However, considering the age of earliest occurrence for HydVac (1 year), it is clearly inducible over a shorter time frame than FCA or neoplasms in these species. Overall, these data suggest that lesions positively associated with age may reflect the cumulative effects of chronic exposure to particular hepatotoxic or hepatocarcinogenic contaminants.

Gender. Gender was not a consistent risk factor for any lesion; the single exception was the predominance of neoplasms detected in male croaker. Only six individuals in this species for which gender data were available were affected with neoplasms, of which five were males. Because of the low number of cases, this relationship is not conclusive. Similar field studies have generally not shown any effect of gender on risk of hepatic lesions in pleuronectids (*21,28*), although recent studies in winter flounder suggest gender-associated differences in neoplasm prevalences (males predominantly affected), possibly as a result of gender-related differences in migratory behavior related to reproductive activities (*31,32*). In contrast, females are more frequently affected with hepatic neoplasms in brown bullhead (*70*), common dab (*36*), and European flounder (*37*) from the North Sea.

Relationships between Chemical Contaminants and Hepatic Lesions

In general, lesions were more likely to occur in fish from sites with higher concentrations of chemical contaminants in sediments. All lesion categories in at least one species showed a chemical class in sediment, stomach contents, liver tissue, or bile as a significant risk factor. The discussion below interprets the toxicological significance, to the extent possible, of the complex associations between lesion types and measures of potential contaminant exposure, actual dietary uptake, hepatic

Table 5. Chemical compounds or classes of compounds in sediment, stomach contents, liver and bile showing significant positive associations by logistic regression ($p < 0.05$) with site-specific prevalences of selected categories of idiopathic liver lesions in starry flounder[a]

Chemical class/compartment	*n*	*p*-value/% total variance			
		FCA	SDN	HydVac	Necrosis
ΣLAHs					
Sediment	33	ns	<0.001/17	<0.001/12	ns
Bile (FACs-L)	35	ns	ns	0.012/3	ns
ΣHAHS					
Sediment	33	ns	<0.001/19	<0.001/13	ns
Stomach contents	15	ns	ns	0.027/3	ns
Bile (FACs-H)	35	ns	0.01/13	0.004/4	ns
ΣDDTs					
Stomach contents	15	0.010/58	ns	<0.001/9	ns
Liver	27	ns	ns	<0.001/25	ns
ΣPCBs					
Sediment	33	ns	0.001/17	<0.001/13	ns
Stomach contents	15	0.028/46	ns	0.03/4	ns
Liver	27	ns	0.041/9	<0.001/29	ns
Hexachlorobenzene					
Sediment	33	ns	ns	<0.001/10	ns
Chlordanes					
Stomach contents	15	ns	ns	<0.001/16	ns
Liver	27	ns	0.013/13	<0.001/28	ns
Dieldrin					
Liver	27	ns	0.001/20	<0.001/36	ns
Metals 1					
Sediment	33	ns	0.004/32	<0.001/12	ns
Liver	19	ns	ns	ns	0.012/36

Abbreviations: FCA, foci of cellular alteration; SDN, specific degenerative/necrotic lesions; Prolif, proliferative lesions; HydVac, hydropic vacuolation; *n*, number of sites; ns, not significant. Neoplasms were not detected in starry flounder, and there were no risk factors associated with proliferative lesions; LAHs, low molecular weight polycyclic aromatic hydrocarbons; HAHs, high molecular weight polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls, FACs-H, aromatic compounds fluorescing at benzo[*a*]pyrene wavelenghts; FACs-L, aromatic compounds fluorescing at naphthalene wavelengths; ES, English sole; SF, starry flounder; WC, white croaker.

[a]Analyses were performed separately while adjusting for mean age and gender ratio (female:male). Table indicates *p*-value, and percent of total variance in lesion prevalence explained by risk factor (reduction in scaled deviance). Neoplasms were not detected in starry flounder, and there were no risk factors associated with proliferative lesions.

bioaccumulation of contaminants, and uptake and metabolism of aromatic compounds. This analysis cannot identify a chemical etiology of the lesions detected among the multiple species examined, but can provide important clues as to which of the many contaminants these species are exposed to may play a role in the development of lesions and an epizootiological basis for definitive cause-and-effect laboratory studies. Because the chemical data for sediments, stomach contents, and liver tissue were derived from composite samples of sediments or fish captured each time a site was sampled, it was not possible to assess the influence of contaminant exposure on disease risk in individual fish. Moreover, the biliary FACs data were in the form of mean site-specific FACs-L and -H levels from individual analyses of 10–12 specimens of a species in a particular year. Consequently, the number of samples for a species available for logistic analysis was relatively low.

Because most lesions detected in this study are inducible over a subchronic to chronic time frame, associations between lesion prevalences and measures of actual exposure to contaminants via dietary uptake, metabolism (biliary FAC levels), and especially hepatic bioaccumulation as a measure of chronic exposure should be regarded as more toxicologically relevant than associations with measures of potential exposure (sediments). However, because a reliable measure of chronic exposure to PAHs was not available for this data set, PAHs in sediments and recent PAH exposure as reflected in mean FAC levels were used as measures of exposure. Another major factor limiting the toxicologic relevance of these associations is the paucity of information relating hepatic tissue burdens of the chemical contaminants measured in this study to pathological effects in fish or other vertebrates.

Neoplasms and preneoplastic FCA. Chemical risk factors for neoplasms were identified primarily in sole, whereas the more prevalent FCA, lesions with the generally accepted potential to develop into hepatocellular neoplasms (*2,10–12, 75,76*) and which significantly co-occur with neoplasms in English sole (*18*), were associated with similar risk factors in all species. The most common risk factors were sediment PAHs, for both neoplasms and FCA in sole, and FCA in croaker, whereas PAHs in stomach contents were risk factors only for FCA in sole. These relationships strengthen those between FAC levels and prevalences of neoplasms in sole and FCA in sole and croaker and are consistent with positive associations shown between hepatic neoplasms and FCA and PAH exposure in other field and laboratory studies with English sole (*3,15, 24,25,39,59*), in studies in other species of wild fish (*69,77,78*) and the hepatocarcinogenicity of genotoxic PAHs in laboratory studies with fish (*5,41,42,79–81*). ΣPAHs in sediment from English sole sites ranged from undetectable at the Nisqually River reference site to 5900 ng/g in Elliott Bay, comparable to levels in previous studies where the relationship between PAH exposure and neoplasms was first established in this species (*15*). The highest level of sediment ΣPAHs from croaker sites was at Hunter's Point (10,300 ng/g), where a significant prevalence of FCA was detected. Because of the lower sample size in sole for stomach contents ($n = 9$) than for sediments ($n = 22$), the absence of either PAH measure in stomach contents as a risk factor for neoplasms is not anomalous considering that contaminant levels in dietary components only reflect exposure at a single time point and may not be reliable indices of chronic exposure. FCA and neoplasms are also apparently inducible over a chronic time frame in this species (*8,18,21*).

ΣPCBs in sediments (≤500 ng/g) and liver (≤11,000 ng/g) were also risk factors for neoplasms in sole, confirming previous findings (*19,20*), as were ΣPCBs in sediments, stomach contents (≤1100 ng/g), and liver for FCA. ΣPCBs in stomach contents (≤490 ng/g) was a risk factor for FCA only in flounder, whereas ΣPCBs was not a risk factor for either lesion in croaker. These concentrations appear to be toxicologically meaningful, independent of their typical covariance with PAHs (*40*), although laboratory studies are clearly needed to confirm this. PCBs are not regarded as genotoxic initiators of hepatic carcinogenesis (*82,83*), do not readily form covalent adducts with DNA, and show minimal mutagenic activity as either individual congeners or complex mixtures (*84,85*). The more highly chlorinated PCB mixtures, especially the toxic coplanar congeners (*83*), are thought to act as epigenetic promoters of hepatocarcinogenesis in rodents (*84–86*) by virtue of cytochrome P450 enzyme induction and consequent hepatotoxicity and stimulation of cell pro-

Table 6. Chemical compounds or classes of compounds in sediment, stomach contents, liver, and bile showing significant positive associations by logistic regression ($p < 0.05$) with site-specific prevalences of selected categories of idiopathic liver lesions in white croaker[a]

Chemcial class/compartment	*n*	*p*-value/% total variance				
		FCA	SDN	HydVac	Prolif	Necrosis
ΣLAHs						
Sediment	43	0.013/28	ns	ns	ns	0.001/17
Bile (FACs-L)	44	0.030/15	0.001/5	<0.001/10	0.001/32	0.003/11
ΣHAHS						
Sediment	43	0.025/23	ns	ns	ns	0.001/21
Bile (FACs-H)	44	ns	0.004/4	ns	0.037/5	ns
ΣDDTs						
Sediment	43	ns	0.001/25	0.033/2	0.013/13	ns
Stomach contents	18	ns	0.001/39	0.001/11	0.001/32	ns
Liver	31	ns	0.001/23	<0.001/20	0.006/17	0.001/9
ΣPCBs						
Sediment	43	ns	0.001/31	0.002/5	0.001/25	0.001/16
Stomach contents	18	ns	0.008/13	0.016/7	0.007/29	ns
Liver	31	ns	0.003/7	<0.001/11	ns	ns
Hexachlobenzene						
Sediment	43	ns	ns	0.004/4	ns	ns
Stomach contents	18	ns	ns	ns	0.035/15	0.037/8
Liver	31	ns	ns	ns	ns	ns
Chlordanes						
Sediment	43	ns	0.005/8	<0.001/12	ns	ns
Stomach contents	18	ns	0.001/37	<0.001/12	ns	ns
Liver	31	ns	ns	<0.001/13	ns	ns
Dieldrin						
Sediment	43	ns	ns	0.004/4	ns	ns
Liver	31	ns	ns	<0.001/9	ns	ns
Metals 1						
Sediment	23	ns	0.001/26	0.001/23	ns	ns
Stomach contents	8	ns	0.008/24	ns	ns	ns
Liver	13	ns	0.003/22	ns	0.015/33	ns
Metals 2						
Liver	13	ns	0.031/15	<0.001/62	ns	ns

Abbreviations: FCA, foci of cellular alteration; SDN, specific degenerative/necrotic lesions; HydVac, hydropic vacuolation; Prolif, proliferative lesions; *n*, number of sites; ns, not significant; LAHs, low molecular weight polycyclic aromatic hydrocarbons; HAHs, high molecular weight polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls, FACs-H, aromatic compounds fluorescing at benzo[*a*]pyrene wavelenghts; FACs-L, aromatic compounds fluorescing at naphthalene wavelengths; ES, English sole; SF, starry flounder; WC, white croaker.

[a]Analyses were performed separately while adjusting for mean age and gender ratio (female:male). Table indicates *p*-value, and percent of total variance in lesion prevalence explained by risk factor (reduction in scaled deviance). No risk factors were associated with neoplasms.

liferation (*83,87,88*). PCBs have also been shown to enhance the formation of FCA in rodent liver (*84,85*). Modulating effects of PCBs on hepatocarcinogenesis in rainbow trout are variable, depending on the timing of administration and the type of initiator used. In aflatoxin-induced hepatic neoplasia in rainbow trout, PCBs are inhibitory to hepatocarcinogenesis (*89,90*), whereas they enhance neoplasm incidence when fed simultaneously with diethylnitrosamine (DEN) (*90*) or have no effect on DEN hepatocarcinogenicity when fed before DEN exposure (*91*). Studies in trout have shown enhancement of 7,12-dimethylbenz[*a*]anthracene-initiated hepatocarcinogenesis when followed by PCB administration (*92*). The existence of ΣPCBs as a risk factor for neoplasms and FCA in sole and FCA in flounder suggests a similar promotional role that may be independent of exposure to and covariance with the genotoxic PAH initiators. However, high prevalences of these lesions are present in sole from a Puget Sound site with sediments containing extremely high levels of ΣPAHs but low levels of ΣPCBs (*16*), and similar lesions are rare in winter flounder from a site in New Bedford Harbor, Massachusetts, with high ΣPCB levels in sediments and liver (3,000 ng/g and 39,000 ng/g, respectively) and relatively low sediment ΣPAHs (*31,32*). Although PCBs may play a promotional role in the development of neoplasms and FCA or function as hepatotoxicants in concert with other contaminants such as PAHs, whether PCB exposure alone can induce these lesions in wild fish is unknown.

ΣDDTs in liver (≤1100 ng/g) and stomach contents (≤290 ng/g) was also a risk factor for neoplasms and FCA in sole; in flounder, only ΣDDTs in stomach contents (≤140 ng/g) was a risk factor for FCA, and ΣDDTs was not a risk factor in croaker. Concentrations in sole liver are regarded as toxicologically significant, and considering the hepatocarcinogenic or promotional effects of these nongenotoxic compounds in rodents (*86,93*) and fish (*79,94*), DDTs and their derivatives should be regarded as potential etiologic factors for neoplasms and FCA in sole, even in light of their covariance with liver ΣPCBs (*40,56*).

Although dieldrin in sediments and chlordanes in stomach contents were potential risk factors for neoplasms in sole, extremely low levels were detected in sediments (≤2 ng/g) and stomach contents (≤50 ng/g), neither was a risk factor in liver tissue, and their levels covaried with sediment levels of other risk factors for neoplasms (e.g., PAHs) (*40*). Therefore, neither are likely etiologic factors. Rainbow trout studies on the co-carcinogenic or promotional effects of dietary dieldrin on aflatoxin-induced carcinogenesis showed a slight but statistically insignificant co-carcinogenic effect, and hepatic lesions were not induced when fed alone (*95*). The carcinogenic potential of this nongenotoxic cyclodiene pesticide is equivocal and controversial in mammalian systems (*96*). However, because of the limited data in fish on the carcinogenic or promotional activity of either of these compounds, they cannot be completely discounted as potential factors in the etiology of neoplasms or FCA in wild fish.

Summed concentrations of copper, zinc, lead, and tin (metals 1) in sediments are also not plausible as etiological factors for these lesions in sole because of the relatively low levels detected (≤420 ng/g), their lack of hepatocarcinogenic potential (*97*), and strong covariance with risk factors of known initiating or promotional potential in hepatocarcinogenesis such as PAHs, ΣPCBs, and ΣDDTs. The absence of metals 1 as a risk factor indicating hepatic bioaccumulation further suggests that these trace elements are not likely factors in the development of neoplasms or FCA. Although a detailed treatment of processes that regulate levels of bioaccumulation of the above elements in fish is beyond the scope of this discussion, teleosts are known to regulate tissue levels of trace elements such as lead, copper, and zinc via mechanisms that alter their uptake, transport, sequestration, detoxification, and excretion, such that simple tissue levels may not accurately measure exposure (*98*). Many toxic metals exhibit rapid turnover in target tissues in mammals, such that no simple relationship exists between administered dose and effective target dose, making it difficult to relate an observed lesion to tis-

sue concentrations or to apply the concept of critical target tissue concentration to the evaluation of toxicity (*99*).

Nonneoplastic proliferative lesions. Risk factors for nonneoplastic proliferative lesions, primarily represented by hepatocellular regeneration, biliary proliferation, presumptive oval cell proliferation (*100*), increased mitotic activity, and cholangiofibrosis, were similar to those for neoplasms and FCA. These lesions are inducible by hepatotoxic/carcinogenic chemicals in mammals [e.g., PCBs (*101*)] and fish (*3,6,7*) and are reliable biomarkers of contaminant exposure in wild fish (*2,18–20, 28,34,44*). Moreover, the first four lesion types represent early compensatory proliferative responses to cytotoxicity after carcinogen exposure (*11*) in the histogenesis of hepatocellular or biliary neoplasia in mammals (*100–102*), acting to fix DNA lesions induced by carcinogen exposure into the genome by cell proliferation.

PAHs were the most common risk factors, but only in sole (sediments, FACs-L and -H, stomach contents) and croaker (FACs-L and -H). ΣDDTs in all compartments was also a risk factor in these species; ΣDDTs strongly covaried with sediment PAHs at English sole sites, but not at croaker sites (*40*). ΣDDTs at croaker sites was ≤670 ng/g in sediments, with extremely high levels detected in liver (26,000 ng/g) and stomach contents (7,600 ng/g) at San Pedro Outer Harbor; ΣDDTs in sole liver was ≤1,100 ng/g. DDT exposure induces liver necrosis in brown trout (*Salmo trutta*) and guppies (*Poecilia reticulata*) (*103*) and hepatocellular vacuolar degeneration, hypertrophy, and necrosis in four species of East Indian fish (*104*). Therefore, ΣDDTs is regarded as a toxicologically meaningful risk factor for these lesions in both species, as possible sequelae to hepatotoxicity. This is especially true for croaker, considering the high bioconcentration factor within liver, a target organ for toxicity of DDTs in mammals (*96*) and fish (*105*).

ΣPCBs was also a risk factor in sole and croaker that covaried with ΣDDTs and PAHs in sediments and stomach contents, and ΣDDTs in sole liver; in croaker, ΣPCBs was correlated with ΣDDTs in all three compartments (*40*). Specifically, ΣPCBs in sediment (≤500 ng/g in sole, ≤430 ng/g in croaker) and stomach contents (≤1100 ng/g in sole, ≤3500 ng/g in croaker) was a risk factor in both species, but liver ΣPCBs was a risk factor only in sole (≤11,000 ng/g). In view of the probable mitogenic effects of PCBs on the liver in mammals (*83*), their promotional potency in hepatocarcinogenesis initiated by certain genotoxic compounds, and the documented induction in mammals of lesion types included within this category such as cholangiofibrosis (*101,106*), exposure to PCBs is regarded as a significant factor in the etiology of these lesions, especially in sole.

Although chlordanes, dieldrin, and metals 1 appeared as risk factors in sole, in view of the relatively low levels in sole stomach contents (≤50 ng/g chlordanes) and sediments (≤3 ng/g chlordanes, ≤2 ng/g dieldrin) and the covariance of metals 1 with more toxicologically plausible risk factors such as PAHs, ΣPCBs, and ΣDDTs, these pesticides and metals 1 are not regarded as meaningful etiologic factors. Dietary uptake of hexachlorobenzene is also not a meaningful risk factor in croaker due to the low levels detected (≤10 ng/g) (*40*).

Specific degenerative/necrotic lesions. Lesions in the SDN category primarily were megalocytic hepatosis and hepatocellular nuclear pleomorphism. These unique lesions are inducible in rodents and fish by exposure to a spectrum of hepatotoxic/carcinogenic or promoting compounds, including PAHs, PCBs, DDTs, and other pesticides, hexachlorobenzene, aflatoxin B_1, and other naturally occurring hepatotoxic compounds, as reviewed in Myers et al. (*18*). These lesions were also induced within 18 months in English sole by multiple injections of an organic-solvent fraction from sediments collected at a creosote-contaminated site (*3*), and have been statistically associated with sediment PAH levels, biliary FACs (*20,59*), and liver ΣPCBs in English sole, as well as biliary FACs in rock sole (*34*). Moreover, megalocytic hepatosis significantly co-occurs with hepatocellular regeneration in individual English sole (*18*). These lesions are regarded as reliable biomarkers of contaminant exposure in wild fish (*2*), including English sole (*18–20,34*) and white croaker (*20,24*).

Risk factors were shown in all three species, again with indices of PAH exposure most frequently identified. Sediment PAHs were risk factors in sole and flounder; this association was further supported by PAHs as risk factors in sole stomach contents. Concentrations of sediment ΣPAHs at English sole sites have been outlined above and are toxicologically significant; for flounder sites, sediment ΣPAH levels ranged from 5 to 10,300 ng/g, the latter level interpreted as toxicologically significant. The role of PAH exposure in the etiology of lesions in this category is further supported in all three species by the association between SDN and at least one biliary FACs measure; both measures were risk factors in sole and croaker.

Consistent with their covariance with sediment PAHs, ΣPCBs was also a risk factor for SDN in all three species; this relationship was supported by ΣPCB bioaccumulation (all species) and dietary exposure (sole and croaker) as risk factors. Concentrations of ΣPCBs in these compartments have been cited above for sole and croaker (except liver levels, ≤15,000 ng/g in croaker) and are interpreted as toxicologically relevant; sediment ΣPCB levels at flounder sites ranged from 1 to 140 ng/g, with hepatic levels ranging from 230 to 7000 ng/g. This level of exposure and extent of hepatic bioaccumulation is also toxicologically meaningful, considering that experimental PCB exposure in mammals (*8,101,106,107*) and fish (*108*) can induce similar hepatic lesions.

ΣDDTs in sediments, stomach contents, and liver were also consistent risk factors for SDN in sole and croaker. Although levels covaried with ΣPCBs within each compartment measured for croaker, ΣDDTs typically accounted for a higher proportion of the intersite prevalence variation for this lesion category than did ΣPCBs, suggesting exposure to ΣDDTs is a more likely factor in the etiology of SDN in croaker. Dietary exposure to and hepatic bioaccumulation of ΣDDTs (up to 7,600 ng/g and 26,000 ng/g, respectively) are certainly high enough to have toxicologic significance. Hepatocellular cytomegaly and karyomegaly have been experimentally induced in mice by exposure to DDT (*8*).

Non-DDT pesticides were potential risk factors for SDN in all species. However, they were not consistently identified among the compartments when consistency would be expected on the basis of significant correlations among the compartments (*40*), and levels detected were quite low. Because of this inconsistency, absence as a risk factor in liver, and the relatively low levels detected (≤3 ng/g, sole sites; ≤15 ng/g, croaker sites, in sediments, ≤50 ng/g, sole; 160 ng/g, croaker, in stomach contents), chlordanes are not a meaningful risk factor in sole and croaker. Hepatic levels of chlordanes and dieldrin were risk factors in flounder, with maximal levels of 330 and 300 ng/g, respectively, indicating a degree of hepatic bioaccumulation. Dieldrin has induced hepatocellular hypertrophy in mammals (*109*) as well as necrosis, and hepatocellular vacuolar degeneration, hypertrophy, and pleomorphism in fish (*110*); it is also considered hepatocarcinogenic in mice (*111*). Chlordanes have also induced liver lesions in mammals (*112*), and fish (*113*), and are epigenetic promoters of carcinogenesis (*86*). The hepatic levels detected suggest that these pesticides could be factors in the genesis of SDN in flounder.

Although metals 1 was a potential risk factor for SDN in all species, because of the strong covariance with more toxicolog-

ically relevant risk factors such as PAHs, ΣDDTs, ΣPCBs (*40*), it is not regarded as meaningful. Metals 2 in liver (≤59 ng/g) as a risk factor for SDN in croaker is also not toxicologically relevant and exists only because of its strong correlation with liver ΣDDTs (*40*).

Necrosis. Chemical risk factors were rarely and inconsistently identified for the nonspecific necrotic lesions, represented primarily by hepatocellular coagulative necrosis. Although this lesion is regarded as a potential biomarker of contaminant exposure in wild fish that needs further confirmation (*2*), inconsistent statistical associations have been shown between this lesion category in several species and contaminant exposure in field studies conducted by our group (*20,21,24*). In this study, the only risk factor common to more than one species (sole and croaker) was hepatic bioaccumulation of ΣDDTs; there were no other risk factors in sole. The hepatotoxicity of DDTs in mammals and fish has been previously discussed. Other risk factors associated with necrosis in croaker were PAHs and ΣPCBs in sediment (at toxicologically significant levels), biliary FACs-H, and hexachlorobenzene in stomach contents. Despite the hepatotoxic potential of hexachlorobenzene (*94*), the low levels detected in croaker stomachs (≤10 ng/g) suggest it is not a meaningful risk factor. Similarly, the toxicologic relevance of metals 1 in liver as an etiologic factor in flounder is questionable because these trace elements are not hepatotoxicants (*97*), and levels were relatively low (≤280 ng/g). Excepting ΣDDTs, no contaminant class was consistently and meaningfully associated with necrosis in more than one species. We therefore regard this lesion as a less reliable biomarker of contaminant exposure, even in species that are apparently susceptible to the effects of such exposure as evinced by the presence of other lesions.

Hydropic vacuolation. Multiple chemical risk factors for HydVac were identified in both affected species, flounder and croaker. We are not aware of an analogous lesion experimentally induced by exposure to identified toxicants in any vertebrate. The lesion is presumably degenerative, with limited proliferative potential (*49,52*), affecting biliary epithelial cells and hepatocytes in winter flounder (*27–29,49–52*), white perch (*114*), starry flounder and rock sole (*34,50*), and white croaker. However, unlike winter flounder (*27,29,49,52*) this lesion in starry flounder and croaker neither co-occurs nor is found in close proximity with neoplasms. The geographic distribution of affected winter flounder (*27,28, 31,32*), rock sole, and starry flounder (*34*) and the statistical association of this lesion with indices of contaminant exposure in winter flounder (*31,32*) and starry flounder (*34*) strongly indicate HydVac is a reliable, specific biomarker of contaminant exposure effects in certain species of wild fish. Chemical risk factors in winter flounder are PAHs, ΣDDTs, and chlordanes (*31,32*); in starry flounder from Puget Sound it is associated with PAH exposure, as reflected in biliary FACs-H levels (*34*). In general, the data here reinforce these relationships. In support of the association shown here between HydVac and levels of biliary FACs-L and FACs-H in starry flounder, potential exposure to PAHs in sediment and dietary uptake were also risk factors. In contrast, the only risk factor of PAH exposure in croaker was levels of biliary FACs-L. This latter finding in croaker is not surprising in view of the lack of correlation between the FACs measures and PAHs in sediments or stomach contents (*40*).

Exposure to CHs occurred most frequently and consistently as risk factors for HydVac in both species. Risk factors of exposure at levels of toxicologic significance were ΣDDTs in sediment (croaker only, ≤670 ng/g), liver (both species, ≤26,000 ng/g in croaker, ≤2,000 ng/g in flounder), and stomach contents (both species, ≤7,600 ng/g in croaker, ≤140 ng/g in flounder), and ΣPCBs in sediment (≤430 ng/g at croaker sites, ≤260 ng/g at flounder sites), liver (≤15,000 ng/g in croaker, ≤7,000 ng/g in flounder), and stomach contents (≤3,500 ng/g in croaker, ≤490 ng/g in flounder) in both species. Actual exposure to non-DDT pesticides, including hexachlorobenzene, chlordanes, and dieldrin, were also consistent risk factors in both species. In flounder, hepatic levels of chlordanes and dieldrin were as high as 330 ng/g and 300 ng/g, respectively, while chlordanes in stomach contents (≤17 ng/g) and hexachlorobenzene in sediment (≤1 ng/g) were quite low. In croaker, non-DDT pesticide risk factors were chlordanes in sediment (≤11 ng/g), stomach contents (≤160 ng/g), and liver (≤1,700 ng/g), dieldrin in liver (≤350 ng/g), and hexachlorobenzene in sediments (≤1 ng/g). Therefore, hexachlorobenzene exposure appears to have no toxicologic significance in croaker. Based on the relatively high hepatic levels of chlordanes and dieldrin, these chemicals cannot be disregarded as toxicologically significant risk factors in either species. However, experimental attempts to induce this lesion in winter flounder by intraperitoneal injection or dietary exposure to high levels of chlordanes were unsuccessful (*28*). Metals 1 in sediments as a risk factor in both species is also probably of no toxicologic significance; it simply is a factor covarying with the PAHs and ΣPCBs (*40*). Similarly, metals 2 in liver as a risk factor in croaker is probably not significant considering the low levels detected (≤60 ng/g) and its strong correlation with hepatic ΣPCBs, a more toxicologically plausible risk factor (*40*).

Therefore, exposure to and uptake of ΣPCBs, ΣDDTs, chlordanes, dieldrin, and PAHs are the most toxicologically relevant risk factors for HydVac consistently shown in both species. These risk factors generally align with the hypothesis (*51*) that this lesion is related to organochlorine exposure and with subsequent hypotheses put forth by Moore (*28,29,49,52*) stressing the potential role of epigenetic promoting compounds in its genesis in winter flounder. However, the potential role of PAH exposure in its etiology should not be minimized, considering the low prevalence (<7%) detected in winter flounder from a site in New Bedford Harbor (*31,32*) that had relatively high sediment levels of PCBs (3000 ng/g) and low to moderate concentrations of sediment PAHs (<2500 ng/g HAHs, <500 ng/g LAHs). Clarification of the role PAH exposure plays in the development of this lesion is further complicated by the unsuccessful experimental induction attempts in winter flounder by chronic dietary exposure to benzo[*a*]pyrene (*28*). Because PCBs and DDTs are readily taken up and bioaccumulated but slowly metabolized, hepatic concentrations of these and other chlorinated compounds may reflect chronic exposure to other covariant contaminants in sediments that are readily metabolized but not bioaccumulated, such as PAHs (*115*). Future incorporation of biomarkers measured in individual fish such as xenobiotic–DNA adducts (*116*), which appear to be persistent and thus estimate chronic exposure to PAHs, should clarify the relative importance of PAHs, and chlorinated hydrocarbons in the development of HydVac and other hepatic lesions. Laboratory studies with starry and winter flounders and croaker exposed to whole sediments, organic-solvent extracts of urban sediments from sites showing high prevalences of this lesion, or model compounds or mixtures are needed to definitively determine how various classes of PAHs and chlorinated hydrocarbons, acting either alone or together, contribute to the genesis of HydVac in teleosts as well as the other lesions detected in this study.

Conclusion

In a comprehensive histopathological and epizootiological study of English sole, starry flounder, and white croaker sampled at sites on the Pacific Coast of the United States, significant prevalences of toxicopathic liver lesions were detected, primarily in fish from urban sites. Many sites showed significantly higher relative risks for at least one lesion type, while controlling for age

and gender. Of greatest importance was the demonstration of positive statistical associations between exposure to contaminant classes and increased lesion risk in all three species. Prevalences of lesions placed in the categories of neoplasms, foci of cellular alteration, nonneoplastic proliferative lesions, specific degeneration/necrosis, and hydropic vacuolation were most commonly associated with exposure to and uptake and metabolism of PAHs, ΣPCBs, and ΣDDTs at exposure or bioaccumulation levels of potential toxicological significance. Chemical risk factors were less commonly identified for nonspecific necrotic lesions. Non-DDT pesticides (chlordanes and dieldrin) were toxicologically relevant risk factors only for specific degeneration/necrosis in a single species (flounder) and for hydropic vacuolation in flounder and croaker.

An important caveat to the utility of these lesions in wild fish as biomarkers of contaminant exposure is that fish age must be accounted for in any analyses comparing lesion risk to site of capture or chemical risk factors. Age is a factor strongly influencing the probability of occurrence of hepatic neoplasms, foci of cellular alteration, nonneoplastic proliferative lesions, and hydropic vacuolation in several species. Gender was not a meaningful risk factor for any lesion.

Not all lesions were detected or consistently associated with exposure to particular contaminants in the species examined, suggesting that not all teleosts respond similarly to exposure to the same classes of toxicants (*74*), and not all lesions identified in this and similar studies can be reliably used as histopathologic biomarkers of contaminant exposure in all species. In English sole, neoplasms, foci of cellular alteration, nonneoplastic proliferative lesions, and specific degeneration/necrosis were meaningfully associated with exposure to PAHs and hepatic bioaccumulation of ΣPCBs and ΣDDTs; nonspecific necrotic lesions were only associated with hepatic bioaccumulation of ΣDDTs. In starry flounder, reliable biomarkers of associated contaminant exposure were hydropic vacuolation (PAH exposure, and hepatic bioaccumulation of ΣPCBs, ΣDDTs, chlordanes and dieldrin), foci of cellular alteration (dietary uptake of ΣPCBs and ΣDDTs), and specific degeneration/necrosis (potential exposure to PAHs and ΣPCBs, and hepatic bioaccumulation of ΣPCBs, chlordanes and dieldrin). All lesions in white croaker except neoplasms were associated with exposure to at least one contaminant class. Specifically, these were foci of cellular alteration (potential and actual PAH exposure), nonneoplastic proliferative lesions (potential and actual PAH exposure, potential exposure to and dietary uptake of ΣPCBs, and potential exposure to and dietary uptake and hepatic bioaccumulation of ΣDDTs), specific degenerative/necrotic lesions (actual PAH exposure and potential exposure to and dietary uptake and hepatic bioaccumulation of ΣPCBs and ΣDDTs), nonspecific necrotic lesions (potential and actual PAH exposure, potential exposure to ΣPCBs, and hepatic bioaccumulation of ΣDDTs); and hydropic vacuolation (actual PAH exposure, potential exposure to and hepatic bioaccumulation of ΣPCBs, ΣDDTs, chlordane and dieldrin).

Because the PAHs, ΣPCBs, ΣDDTs, chlordanes, dieldrin, and metals 1 typically covaried in sediments, stomach contents, or liver/bile (PAHs and their metabolites), it is clear that bottomfish in this study are exposed simultaneously to a complex mixture of contaminants. Therefore, quantification of the relative contribution of any significant chemical risk factor with respect to the etiology of hepatic lesions is not possible solely on the basis of epizootiological data. The existence of the ΣPCBs and ΣDDTs as strong risk factors for several classes of hepatic lesions in all three species may be due to their well-known bioaccumulation and persistence in fish tissue, thus serving as general indices of long-term exposure not only to CHs but other covarying contaminants such as PAHs (*115*). Ongoing field studies that measure these contaminants (including congener-specific coplanar PCBs) and biomarkers of contaminant response (e.g., hepatic aryl hydrocarbon hydroxylase activity, xenobiotic-DNA adducts) in individual fish also assessed histopathologically will further enhance our ability to identify relationships between environmental contaminants and toxicopathic hepatic lesions in native fish species, as well as to account for the confounding effects of fish migration. Ultimately, laboratory exposure studies are essential to conclusively establish the roles of specific toxicants in the etiology of pollution-associated disease in wild fish. However, the existence of toxicologically plausible and statistically significant and consistent relationships between these risk factors and hepatic lesions provides strong epizootiological evidence supporting the role of chemical contaminants in the etiology of these lesions and clearly indicates their utility as biomarkers of contaminant exposure effects in native fish species sampled in biomonitoring studies.

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