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RSET Issue Paper: Proposed Sediment Screening Level for PAHs for Protection of Salmonids

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INTRODUCTION

Existing sediment standards used by the Regional Sediment Evaluation Team (RSET) to evaluate suitability of dredged materials are primarily derived for protection of benthic invertebrates. However, fish respond to some contaminants in sediment in a more unique way that may not be accounted for in benthic invertebrate tests, and impacts to fish can occur at lower concentrations than those considered protective of invertebrates. Petroleum aromatic hydrocarbons (PAHs) are a group of compounds that can cause effects in some fish at comparatively lower concentrations than invertebrates, and include effects that are not typically measured in invertebrates such as liver lesions, DNA damage, changes in lipid content that have impacts to growth, and changes in disease prevalence (Johnson et al. 2002). Because dredging activities can occur in habitats where threatened or endangered juvenile salmon species are present, the National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (FWS) have concerns that existing standards for PAHs may not be protective of juvenile salmonids. This white paper builds on research conducted on English sole by the National Oceanic and Atmospheric Administration (NOAA) Fisheries Science Center in Seattle, Washington (Johnson et al. 2002) and other data specific to salmonids to derive sediment screening levels that would be protective of individual juvenile salmonids in both freshwater and marine systems. This paper summarizes the pathways for PAHs from sediments into fish, describes adverse effects and response of fish to exposure, and proposes a sediment level for PAHs that would be considered protective of salmonids. NMFS and FWS recommend that RSET agencies consider these proposed sediment PAH values when evaluating dredging and disposal activities where juvenile salmonids or other threatened and endangered fish are present.

SENSITIVITY OF FISH TO PAHS

Summary of dietary exposure and effects to fish

Fish are particularly sensitive to PAH toxicity because they metabolize PAHs to toxic and mutagenic intermediates, and are susceptible to the carcinogenic and mutagenic effects of PAHs (Varanasi et al. 1987, Meador et al. 1995). A number of effects have been associated with dietary exposure to PAHs (Table 1). Exposure to PAHs may affect juvenile salmon growth (Carls et al. 1996, Meador et al. 2006), a particularly significant effect, as growth rate is a good predictor of survival of juvenile salmon in the first year of life (Zabel and Achord 2004). Carls et al. (1996) showed reduced growth in juvenile pink salmon exposed to dietary PAHs at a concentration of 0.14 mg/kg bw/day, which is equivalent to 0.9[1] mg/kg wet weight (ww) in diet. Meador et al. (2006) observed changes in physiological measurements associated with growth

[1] Based on food ingestion rate of 16% body weight for juvenile salmon (Meador et al. 2008)

and energy balance at a dose of 2.3 mg/kg body weight (bw)/day (14[1] mg/kg ww in diet). Moreover, the same study showed greatly increased variability in fish weight at a still lower dose of 0.7 mg/kg bw/day (4.4[1] mg/kg ww in diet), which could be significant for the survival of some individuals in the population.

PAHs can also affect the salmon immune system, compromising disease resistance. Arkoosh et al. (1991, 1994, 1998, 2001) demonstrated that juvenile Chinook salmon exposed to PAHs in estuaries of Puget Sound, or treated with PAHs in the laboratory setting, had a suppressed secondary immune response and reduced survival in disease challenge. A similar study by Bravo et al. (2011) reported alteration in a variety of biochemical markers and decreased survival in disease challenge in rainbow trout fed a diet of 0.66 mg/kg bw/day (6.2 mg/kg ww in diet).

In addition to studies with juvenile salmon, a laboratory exposure study by Rice et al. (2000) showed decreased growth in juvenile English sole fed polychaete worms with a PAH concentration of 2.3 mg/kg ww. After an exposure period of 12 days, the growth rate (measured as change in weight) was markedly lower (0.05 to 0.1% per day) in exposed fish than in control fish (1.1 to 1.2% per day).

Stomach content PAH concentrations and lesion prevalences in English sole collected as part of the National Benthic Surveillance Project (NBSP; Myers et al. 1994, 1998, 2003; Brown et al. 1998) have also been used to generate a hockey stick model estimating a dietary threshold concentration increased lesion prevalence (Figure 1, Table 1). This approach yielded threshold dietary benchmarks of 0.2 to 0.6 mg/kg ww total PAHs in stomach contents for degenerative lesions and neoplasms, with the upper confidence limits of these estimates in the 1.5 to 3.0 mg/kg ww range. These dietary PAH concentrations in English sole approach the concentration range (listed previously) associated with injury to salmon. Studies in the lower Willamette River have shown that juvenile salmon had higher concentrations (total PAH concentration of 2.5 mg/kg ww) in stomach contents than in some of the above referenced studies where effects have been observed, and well above concentrations associated with liver neoplasms and impaired growth in English sole. Mean total PAH concentrations for all the juvenile salmonid stomach contents samples in the lower Willamette and Columbia Rivers was 3.5 mg/kg ww, with the highest concentrations from the Columbia/Willamette River confluence at 54 mg/kg ww (Yanagida et al. 2012; L. Johnson, unpublished data).

Although dietary exposures indicate salmonids, as well as other fish, are sensitive to PAHs and dietary thresholds have been calculated for juvenile Chinook salmon (Meador et al. 2006), calculating sediment benchmark values based on dietary exposures is complex and may be less informative than comparing direct toxicity values from fish exposed to PAHs in sediment.

Summary of sediment exposure and effects to fish

A number of fish species have been evaluated for effects due to exposure to PAHs in sediment, both in the laboratory and field. For example, 50 to 60% of brown bullhead evaluated at some sites in Chesapeake Bay showed liver neoplasms with dry weight (dw) sediment PAH concentrations ranging from 5,000 to 28,000 ng/g (Pinkney et al. 2001, 2004a, 2004b, 2009). Similarly, 25% of brown bullhead in the Trenton Channel in the Detroit River had liver

neoplasms when PAH concentrations ranged from 84 to 535,000 ng/g dw in surface sediments (Leadley et al. 1998) with a mean concentration of 39,000 ng/g dw (MDEQ and EPA 2010). In other fish species, induction of PAH-metabolizing enzymes, reduced growth, embryo mortality, and altered stress response have been reported at sediment PAH concentrations ranging from 500 to 5,000 ng/g dw (Table 2). Benchmarks derived from studies with English sole have perhaps the most robust, consistent, and clear associations with exposure to PAHs based on field and laboratory studies as compared with other fish species, including freshwater fish species. English sole liver lesions associated with PAH sediment benchmarks include specific degeneration/necrosis (SDN), proliferative lesions (hepatocellular regeneration, oval cell proliferation, bile duct hyperplasia), preneoplastic foci of cellular alteration (FCA), and liver tumors or neoplasms (Tables 2 and 3). SDN is a lesion category that includes nuclear pleomorphism and megalocytic hepatosis (also referred to as hepatic megalocytosis), and is a type of liver degeneration caused by exposure to contaminants, including PAHs. The proliferative lesions, listed above, represent a compensatory cell growth and proliferation response to liver degeneration caused by exposure to contaminants including PAHs (Myers et al. 1987). FCA are areas of liver cell alteration that are considered to be precursors of liver neoplasms. All of these lesions show strong and consistent correlations with PAHs in sediments, bile, and stomach contents in English sole in multiple field studies (Myers et al. 1992, 1994, 1998, 2003) and SDN, proliferative lesions, and FCA have also been produced in the livers of English sole exposed to PAHs in the laboratory (Schiewe et al. 1991).

In addition to studies of liver disease, two laboratory exposure studies show decreased growth in juvenile English sole exposed to PAHs (Kubin, 1997, Rice et al. 2000). Although neither of these experiments was designed specifically to identify effects thresholds or model dose-response relationships, the results provide information on PAH exposure levels associated with growth impairment in sole. Kubin (1997) observed a 19% reduction in growth rate, measured either in length or weight, in juvenile English sole exposed for six months to sediment containing 4,000 ng/g dw total PAHs, but no effect at levels of 2,000 ng/g dw and below, suggesting an effect threshold in the 2,000 to 4,000 ng/g dw range. Actual threshold effect concentrations are likely lower because uptake of PAHs in this experiment was from sediment and water only; in the natural environment, substantial exposure would also occur through the diet. A study by Rice et al. (2000) detected similar effects of PAHs on growth of juvenile English sole. The findings showed significantly reduced weight in juvenile English sole fed polychaete worms reared on sediments containing 3,000 to 4,000 ng/g dw of PAHs, after an exposure period of only 12 days. The growth rate was markedly lower (0.05 to 0.1% per day) in exposed fish than in control fish (1.1 to 1.2% per day). Tables 2 and 3 summarize effects in various fish, particularly English sole, along with associated sediment PAH concentrations.

Summary of metabolites of PAHs in bile and effects to fish

Because fish rapidly biotransform and therefore accumulate PAHs to only a limited extent, a useful tool for measuring exposure in fish is through concentrations of PAH metabolites in bile (Krahn et al. 1986, Beyer et al. 2010). Bile retrieved from the gallbladder of exposed fish contains PAH metabolites and alkylated PAHs, and these compounds can be quantified using fluorescent aromatic compounds (FACs; Krahn et al. 1986; Meador et al. 2008). Results are typically reported as naphthalene, phenanthrene (PHN), and benzo[a]pyrene (BaP) equivalents. Though not extensive, there are data linking bile metabolite levels with various types of injury in

fish. A number of case studies in wild fish that have linked the occurrence of hepatic neoplasms and neoplasia-related toxicopathic liver lesions to PAH exposure, as measured by PAH metabolites in fish bile. In English sole, this association, documented by multivariate analytical techniques such as stepwise logistic regression, has held true for over 12 separate multi-site, multiyear field studies with both adult and subadult English sole sampled from Puget Sound, as well as the Pacific coast from southern California to Alaska. Moreover, these studies involved multiple investigators, including the Northwest Fisheries Science Center, the Washington Department of Fish and Wildlife, and Environment Canada (Myers et al. 2003). Similarly, in field studies conducted as part of the NBSP in the 1980s, significant ($p < 0.05$) positive associations were found between the prevalence of SDN in starry flounder and levels of PAH metabolites in bile (Myers et al. 1994). In brown bullhead from the contaminated Anacostia River and the Tuckahoe River reference site, PAH-like metabolites in bile were a significant predictor variable for the occurrence of liver neoplasms in brown bullhead (Pinkney et al. 2009). Elevated levels of PAH metabolites in bile have also been linked to reduced growth (Rice et al. 2000), reduced levels of reproductive hormones (Johnson et al. 1998, Sol et al. 2000), and changes in lipid content and weight (Meador et al. 2006). Several of these studies are listed in Table 4. Using these and related data, Meador et al. (2008) proposed a threshold (no-effect) value of 2 µg FACs-PHN/mg bile protein for toxic effects of PAHs.

A study by Cormier et al. (2000) provides perspective on the significance of bile metabolite levels in white sucker (*Catostomus commersonii*), another freshwater species related to sucker species such as the largescale sucker (*Catostomus macrocheilus*), widespread in the Pacific Northwest. In this study Cormier and colleagues reviewed data on BaP and NPH-type metabolites in bile of white sucker sampled throughout the United States as part of the Environmental Protection Agency (EPA)'s Regional Environmental Monitoring Assessment Program (REMAP) and the Ohio Environmental Protection Agency Biomonitoring Program (OEPA). Based on the distributions of bile metabolite concentrations, they established exposure criteria of 0.4 µg/mg protein for BaP and 60 µg/mg protein for NPH from the REMAP study, and 0.5 and 80 µg/mg protein from the OEPA study – values that are not significantly different. These values represent the 95th percentiles of the concentrations obtained as part of these studies, which include a large number of sites from both reference and contaminated areas, and so would indicate a relatively high level of exposure. From the same data sets, 25th percentile value were also calculated of 0.117 to 0.178 µg/mg protein for BaP and 22.6 to 26.6 µg/mg protein for NPH, depending on the data set used. The upper values might be considered as representing high exposure concentrations where injury was likely, while the lower values might be considered as indicating relatively low exposure and minimal risk.

For English sole, using limited data collected as part of the Puget Sound Ambient Monitoring Program (PSAT 2007) and Hylebos injury assessment studies (Collier et al. 1998), the estimated threshold for lesion occurrence for BaP metabolites is 0.46 µg/mg bile protein, which is very consistent with the white sucker criterion for a PAH-contaminated site (Figure 2). The threshold for lesion occurrence for FACs-PHN is 14 µg/mg bile protein, with a lower confidence interval of 10 µg/mg bile protein. This is substantially higher than the threshold suggested by Meador et al (2008), and also somewhat higher than the 5 to 10 µg/mg protein concentration at sites where English sole lesion prevalences were elevated in Puget Sound (PSAT 2007). However, these estimates may change somewhat with incorporation of additional data. There were not sufficient

data on protein-adjusted FACs-NPH to estimate a threshold for lesion occurrence using hockey stick regression.

Based on data collected from Puget Sound English sole as part of the Puget Sound Ecosystem Monitoring Program (PSEMP; see PSAT 2007), the NPH benchmarks of 22 µg/mg bile protein and 60 µg/mg bile protein and BaP benchmarks of 0.117 and 0.4 µg/mg protein from Cormier et al. (2000) would accurately represent fish with relatively low and relatively high PAH exposure. The 0.4 µg/mg protein benchmark is consistent with BaP metabolite levels reported in English sole from urban sites in Puget Sound where liver disease and reproductive injury have been documented (Johnson et al. 2008). Similarly, English sole with FACs-PHN levels near or below the 2 µg/mg bile protein benchmark discussed in Meador et al (2008) would be representative of fish from minimally contaminated sites with little PAH exposure (PSAT 2007).

The bile benchmark of 2 µg FACs-PHN/mg bile protein suggested by Meador et al. (2008) is consistent with bile metabolite levels measured in juvenile Chinook salmon from the Pacific Northwest. For example, in the Lower Columbia River, Yanagida et al. (2012) report FACs-PHN levels at relatively uncontaminated reference sites in the 1 to 2 µg/mg protein range, while levels at urban sites are in the 3 to 5 µg/mg protein range. Reported levels in juvenile Chinook salmon from Puget Sound are even higher, with values above 10 µg/mg protein (Olson et al. 2008; Kelley et al. 2011). FACs-BaP levels are generally lower than the exposure benchmarks proposed by Cormier et al. (2000) for white sucker, however, with levels typically ranging from 0.03 to 0.05 µg/mg protein at minimally developed sites to 0.3 to 0.4 µg/mg protein at urban or industrial sites (Olson et al. 2008; Yanagida et al. 2012). The same is true of the FACs-NPH benchmarks 22 and 60 µg/mg bile protein, as reference site values are below 10 µg/mg bile protein, and even at the most contaminated sites in Puget Sound and the Columbia River are in the 40 to 50 µg/mg bile protein range (Yanagida et al. 2012; Olson et al. 2008).

SEDIMENT THRESHOLDS RELATED TO FRESHWATER FISH

For English sole, the benchmarks for liver lesions are considered highly robust because they are based on a large volume of reliable data, and also because separate analyses of datasets from the NBSP and the Puget Sound Assessment and Monitoring Program yield very similar threshold concentrations for all lesion categories (Johnson et al. 2002, Myers et al. 2003). Moreover, the cause-and-effect relationship between PAHs and these lesions has been clearly demonstrated in laboratory exposures, and strong and consistent associations with PAHs have been observed in multiple field studies (Schiewe et al. 1991, Myers et al. 2003).

While benchmarks associated with English sole are quite robust, nonetheless it is a saltwater fish species that does not exist in many freshwater sites. Consequently, the Northwest Fisheries Science Center has developed hockey stick regression models relating sediment PAHs and liver lesion prevalence similar to those developed for English sole for two additional species found in the Pacific Northwest including the lower Willamette River: starry flounder (*Platichthys stellatus*) and brown bullhead (*Ameiurus nebulosus*; see Figure 3), as well as for the mummichog (*Fundulus heteroclitis;* see Figures 3 to 5). The mummichog is an east coast species, but belongs to the same genus as the banded killifish (*Fundulus diaphanus*), which is found in some northwest waters such as the lower Willamette River. These benchmarks, as listed in Tables 2 and 3, indicate that PAHs cause degenerative lesions in species other than English sole at similar

concentrations (Lomax et al. 1994, Johnson et al. 2002). Winter flounder (*Pseudopleuronectes americanus;* see Figure 6) an additional saltwater species found, along the Northeast coast of the United States also has benchmarks for liver lesion induction at concentrations in the same range. This information collected on effects from exposure to PAHs on fish indicates that English sole is a representative sentinel fish species: We would expect to see similar degrees of effect in other fish species at PAH concentrations similar to the benchmarks presented for English sole.

These benchmarks are shown in Figure 7 (see also Table 3) in comparison with sediment quality benchmarks, most of which are based on effects in benthic invertebrates, illustrating the sensitivity of fish to PAHs and the similarity of responses among fish in relation to sediment concentrations.

RECOMMENDATIONS

The information outlined above on the effects of PAHs on fish clearly shows that these species are often more sensitive than invertebrates to these compounds, and that a sediment evaluation framework focused on benthos may not be protective of fish. Using the data collected on the relationship toxicant-related injury and PAH concentrations in sediments, fish diets, and fish bile, we have developed a modified evaluation framework for PAHs and fish (see Figures 8 and 9).

For such a framework, sediment screening levels and benchmarks are needed. The approach of selecting a low percentile of a series of representative toxicity studies to determine a concentration for protection is well-established, and has been employed by the EPA for determining national water quality criteria (Stephan et al. 1985) as well as in studies to identify tissue residue values protective of juvenile salmon (Meador et al. 2002). If we apply such an approach to the studies associating biological effects in fish with sediment PAH concentrations (Table 2), the 10th percentile value of all studies included is 640 ng/g dw while the 25th percentile value is 1,800 ng/g dw. Values below the 10th percentile are considered highly protective for juvenile salmonids migrating through urban estuaries, as well a resident fish species, while those below the 25th percentile would afford protection against the majority of adverse effects. These benchmarks are consistent with those of 1,000 to 2,000 ng/g dw total PAHs recommended in Johnson et al. (2002) and by Wolotira (2007). They are also very similar to the Consensus Threshold Effects Concentrations below which invertebrates are not expected to suffer from adverse effects (MacDonald et al. 2000). We suggest adopting a value in this range as a screening level value for protection of estuarine and freshwater fish, including juvenile salmon.

If the recommended sediment screening values are exceeded, then there are several alternative testing options (see Figures 8 and 9). First, a fish bioassay test could be employed in which a representative fish species such as rainbow trout (*Oncorhynchus mykiss*) is exposed to the test sediment, and relevant effects or exposure measured. Measurements could include growth, mortality, and PAH metabolite levels in bile, which can be compared to threshold benchmarks of concern. Meador et al. (2008) has already suggested a no-effect benchmark of 2 µg FACs-PHN/mg protein for PAH metabolites in fish bile. Cormier et al. (2000) suggest values of 0.4 µg

FACs-BAP/mg protein and 60 µg FACs-NPH/mg protein to denote impaired waters, with 0.12 µg FAC-BaP/mg protein and 22 µg FACs-NPH/mg protein associated with low exposure. If we apply the approach used for the sediment and diet data to the studies listed in Table 4, relating effects in fish to PAH metabolite levels in bile, the 10th percentile value of all studies included is 4.3 µg FACs-PHN/mg bile protein, while the 25th percentile value is 4.8 µg FACs-PHN/mg bile protein, suggesting additional potential suitable benchmarks in a comparable range. Although the fish bioassay approach would be the most direct way to measure availability and toxicity of PAHs in sediment to fish, there currently are no standardized fish bioassay protocols (Corps 2009), so any proposed testing would need to be approved by local sediment review groups.

Alternatively, a standard invertebrate bioassay or bioaccumulation test with microanalysis of tissue after exposing test invertebrates to PAH-contaminated sediment (e.g., Inouye and Lotufo 2006, Jones et al. 2006, Millward et al. 2007) could be used to examine PAH accumulation in a representative invertebrate prey species for the fish species of concern, and the resulting prey PAH concentrations could be compared to a dietary benchmark generated from data such as the studies listed in Table 1. Failure to pass the bioaccumulation or bioassay prey tissue test would typically lead to a determination that the material is unsuitable for unconfined aquatic disposal. Using studies associating biological effects in fish with PAHs in the diet (Table 1) to obtain benchmark values, the 10th percentile value of all studies included is 0.81 mg/kg ww while the 25th percentile value is 1.95 mg/kg ww. The average total PAH concentration in stomach contents of English sole collected from urban sites along the Pacific coast as part of the NBSP ranged from 7 to 40 mg/kg ww, while the average for sole from relatively uncontaminated reference sites ranged from 0.13 to 1.75 mg/kg ww (see Brown et al. 1998). For juvenile Chinook salmon from Pacific Northwest sites, total PAH concentration in stomach contents of salmon at relatively uncontaminated reference sites were typically less than 0.5 mg/kg ww. At other sites, levels ranged from 1 to 2 mg/kg at sites with moderate development to 7 to 14 mg/kg ww at urban sites (Johnson et al. 2007, Yanagida et al. 2012). Thus, benchmarks in the range above are reasonable as compared with actual exposure in field-collected fish, though additional data, when available, on dietary PAHs and biological effects should be included for a more rigorous analysis.

One drawback of the bioaccumulation testing approach with fish prey is that the organisms currently approved for bioaccumulation testing may not be appropriate surrogates for the prey of salmonids or other fish species of concern. The Ocean Testing Manual (EPA/Corps 1991) and the Inland Testing Manual (EPA/Corps 1998) provide information on bioaccumulation tests for freshwater and marine sediments (Corps 2009). For marine sediments, a 28-day bioaccumulation test should be conducted with an adult bivalve (*Macoma nasuta*) and an adult polychaete (*Nereis virens, Nepthys,* or *Arenicola marina*). These species might be reasonable surrogates for a saltwater fish species such as English sole, but not for estuarine salmonids. For freshwater sediments, the test should be conducted with the oligochaete (*Lumbriculus variegatus*) and a second species to be determined at the time of testing. The freshwater amphipod *Diporeia,* is included as an acceptable freshwater species. This amphipod is found in the Great Lakes, where it is a component of the diet of juvenile Chinook salmon, so it might be a suitable surrogate for the amphipod species consumed by juvenile salmonids in the Pacific Northwest. Selection of additional approved species for freshwater bioaccumulation testing is in progress, so additional alternatives may soon be available. A Biological Testing Subcommittee White Paper has addressed some issues related to bioassays and bioaccumulation testing for the

protection of fish, and may provide suggestions for alternative test species.

As in the Sediment Evaluation Framework (Corps 2009), several special evaluations might also be called for (Figure 9). For example, in lieu of bioassays or bioaccumulation testing, dietary and bile benchmarks could be used as part of a field assessment, to determine if fish from area of concern actually showed sufficient exposure to PAHs to put them at risk of injury. If water quality effects at the point of dredging are anticipated, then elutriate testing or porewater testing using solid phase microextraction techniques (see King et al. 2003, IRTC 2011) for PAHs or other evaluations would be indicated to evaluate bioavailability of PAHs. If listed salmonids are present in a freshwater area of concern, then current procedures already indicate inclusion of rainbow trout as one of the test species for elutriate bioassay testing (Corps 2009). Analysis of bile samples from exposed fish in these tests for comparison to bile metabolite benchmarks would be a useful procedure for risk evaluation if PAHs were among the contaminants of concern at a site. Finally, if sediment removal uncovered more contaminated sediments at depth, or other problems arose triggering evaluation of new surface materials to evaluate dredging residuals, then suggested screening levels for PAHs could be applied in evaluation of these sediments when exposure of listed fish species a concern.

Table 1. Studies documenting the relationship between dietary ∑PAH concentration and biological effects in fish.

Species	Endpoint	Dose (ppm fish wt/day)	Concentration in food (mg/kg ww)	Reference
English sole	Liver lesions	-	0.2-0.6[1]	This paper
Pink salmon	Reduced growth rate	0.14	0.9[2]	Carls et al. 1996
English sole	Reduced growth rate	0.12	2.3[3]	Rice et al. 2000
Rainbow trout	Decreased disease resistance	0.66	6.2[3]	Bravo et al. 2011
Chinook salmon	Increased growth variability	0.7	3.8[3]	Meador et al. 2006
Chinook salmon	Changes in enzymes related to lipid metabolism	2.3	12[3]	Meador et al. 2006
Chinook salmon	Changes in lipid content	6.1	32[3]	Meador et al. 2006
Chinook salmon	Reduced weight	18	95[2]	Meador et al. 2006
Mean ± SD		19.1 ± 32.4	**10th percentile**	0.81
Geometric mean		5.7	**25th percentile**	1.95
Median		5.0		

[1]Represents threshold value estimated from hockey stick regression, not concentration where a significant effect is first observed.

[2]Calculated from the equation dose = [prey]*dietary ingestion rate, which is estimated to be 16% of body weight per day, based on studies with juvenile salmon (see Meador et al. 2008).

[3]Measured concentration in food.

Table 2. Studies documenting the relationship between ∑PAH concentration in sediments and biological effects in fish.

Species	Compound	Endpoint	Concentration (ng/g dry wt)	Reference
Senegalese sole	PAHs in sediment	EROD/GST induction	~500	Jiminez-Tenorio et al. 2008
English sole	PAHs in sediment	Proliferative lesions in liver	230	Horness et al. 1998
English sole	PAHs in sediment	PAH-DNA adducts in liver	290	Johnson et al. 2002
English sole	PAHs in sediment	One of more toxicopathis lesions in liver	620	Horness et al. 1998
Winter flounder	PAHs in sediment	Hydropic vacuolation in liver	430	This paper
English sole	PAHs in sediment	Spawning inhibition, reduced egg and larval viability	630	Johnson et al. 2002
Winder flounder	PAHs in sediment	Foci of alteration in liver	1,000	This paper
Senegalese sole	PAHs in sediment	Degenerative liver lesions	1,100	Costa et al. 2009
Turbot	PAHs in sediment	Reduced growth; lipid depletion	2,000-3,000	Kerambrum et al. 2012
Pink salmon eggs	PAHs in weathered oil	Embryo mortality	2,800	Murphy et al. 1999
Pink salmon embryos	PAHs in weathered oil	Embryo mortality	3,800-4,600	Heintz et al. 1999
Brown bullhead	PAHs in sediment	Liver neoplasms	3,400	This paper
White perch	PAHs in sediment	Altered stress response	3,000-4,000	Hontela et al. 1995,1995
Mummichog	PAHs in sediment (BaP only)	EROD activity in the intestine	3,500	Couillard et al. 2009
English sole	PAHs in sediment	Inhibited ovarian growth	4,000	Johnson et al. 2002
Chinook salmon juveniles	PAHs in sediment	Reduced growth	> 5,000	Casillas et al. 1995, 1998
Chinook salmon juveniles	PAHs in sediment	Reduced growth	> 5,000	Arkoosh et al. 1998
Mummichot	PAHs in sediment	Liver neoplasms	16,200	This paper
Zebrafish	PAHs in sediment	Developmental abnormalities	27,000	Raimundo et al. 2014
Zebrafish	PAHs in sediment	Mortality	78,000	Raimundo et al. 2014
Mean ± SD	7,500 ± 1,500	**10th percentile**	640	
Geometric mean	3,000	**25th percentile**	1,800	
Median	3,500			

Table 3. Summary of PAH concentrations in sediment associated with effects and fish and invertebrates.		
PAH concentration (ng/g dw sediment)	**Description**	**Reference**
<1,000	Fish proliferative lesions (230 ng/g dw) –English sole liver; strong correlations with PAH concentrations in sediments and sole diets and PAH metabolites in fish bile (laboratory and field exposures). PAH-DNA adducts (288 ng/g dw) in English sole liver; indicator of binding PAHs to DNA and associated with DNA damage and risk of mutation and a risk factor for development of neoplasms and other liver lesions (induced in laboratory). Toxiopathic lesions (620 ng/g dw)- includes neoplasms, foci of cellular alteration, specific degeneration/necrosis, proliferative lesions in English sole liver caused by exposure to PAHs (strong correlation in sediments in sole diets, and metabolites in bile) and produced in laboratory. Fish inhibited spawning and reduced egg and larval viability (630 ng/g dw)- based on English sole exposed at PAH-contaminated sites in field brought to the laboratory and induced to spawn hormonally.	Horness et al. 1998 Johnson et al. 2002 Wolotira 2007
1,000–5,000	Level (1,000 ng/g dw) for multiple sublethal effects in English sole and 5 times increase in DNA damage in liver. Invertebrate value (1,600 ng/g dw)- below which effects to invertebrates not expected (Consensus Threshold Effects Concentration). Threshold for precursor lesions in English sole, starry flounder, brown bullhead, and mummichog. Threshold for DNA adducts in liver of mummichog. Strong correlations with PAH in sediment and English sole diet and bile. Inhibited gonadal growth (4,000 ng/g dw)-decreased likelihood of normal ovary development in sole.	Johnson et al. 1988; 1999; 2002; unpublished data Casillas et al. 1991 Wolotira, 2007 MacDonald et al. 2000
> 5,000–10,000	Higher degree of sublethal effects on English sole (approximately 25% lesion rate in English sole). For invertebrates-minimum concentration (7,900 ng/g dw) above which mortality effects are observed in all tests on echinoderms, and minimum concentration (8,100 ng/g dw) above which mortality effects are observed in all tests on neanthes.	

> 10,000–22,800	Higher degree of sublethal effects on English sole (40% lesion rate, clear declines in percentage of English sole that undergo normal ovarian development and spawning, egg viability substantially reduced) and lesions expected in less sensitive fish (brown bullhead; 16% lesion rate). Effects to oysters (17,000 ng/g dw) and other invertebrates observed.	Wolotira, 2007 WDOE 1996 Baumann et al. 2000 Pinkney et al. 2004a
> 22,800–69,000	Consensus Probable Effects Threshold (22,800 ng/g dw) on benthic invertebrates (harmful effects likely to be observed). Minimum concentration (69,000 ng/g dw) above which mortality effects are observed in all tests on amphipods.	MacDonald et al. 2000 WDOE, 1996
> 69,000–100,000	All apparent effects thresholds exceeded (amphipod and benthic community). Increased percentage of lesions in English sole.	Johnson et al. 2002 Wolotira, 2007
> 100,000	High rates of sublethal effects in English sole (~ 75% lesion rate and fecundity reduced by ~ 50%).	Johnson et al. 2002 Wolotira 2007

Table 4. Studies documenting the relationship between PAH metabolites in bile and biological effects in fish.

Species	Endpoint	Dose (ppm fish wt/day)	Bile metabolites FACs-PHN (µg/mg bile protein)	Reference
English sole	Increased liver lesion risk	-	5[a]	PSAT 2007
English sole	Reduced growth rate	0.12	4.7[b]	Rice et al. 2000
Pink salmon	Reduced growth rate	0.14	4.8[b]	Carls et al. 1996
Rainbow trout	Decreased disease resistance	0.66	5.5[b]	Bravo et al. 2011
Chinook salmon	Increased growth variability	0.7	3.5[b]	Meador et al. 2006
Dolly varden trout	Reduced reproductive hormones	-	5[a]	Sol et al. 2000
Chinook salmon	Changes in enzymes related to lipid metabolism	2.3	6.3[a]	Meador et al. 2006
Chinook salmon	DNA damage in gill tissue	-	11[a]	Kelley et al. 2011
Chinook salmon	Changes in lipid content	6.1	12[a]	Meador et al. 2006
Chinook salmon	Reduced weight	18	36[a]	Meador et al. 2006
Mean ± SD	9.2 ± 7.0	**10th percentile**	4.3	
Geometric mean	5.5	**25th percentile**	4.8	
Median	5.0			

[a]measured value

[b]estimated from equation in Meador et al. 2008

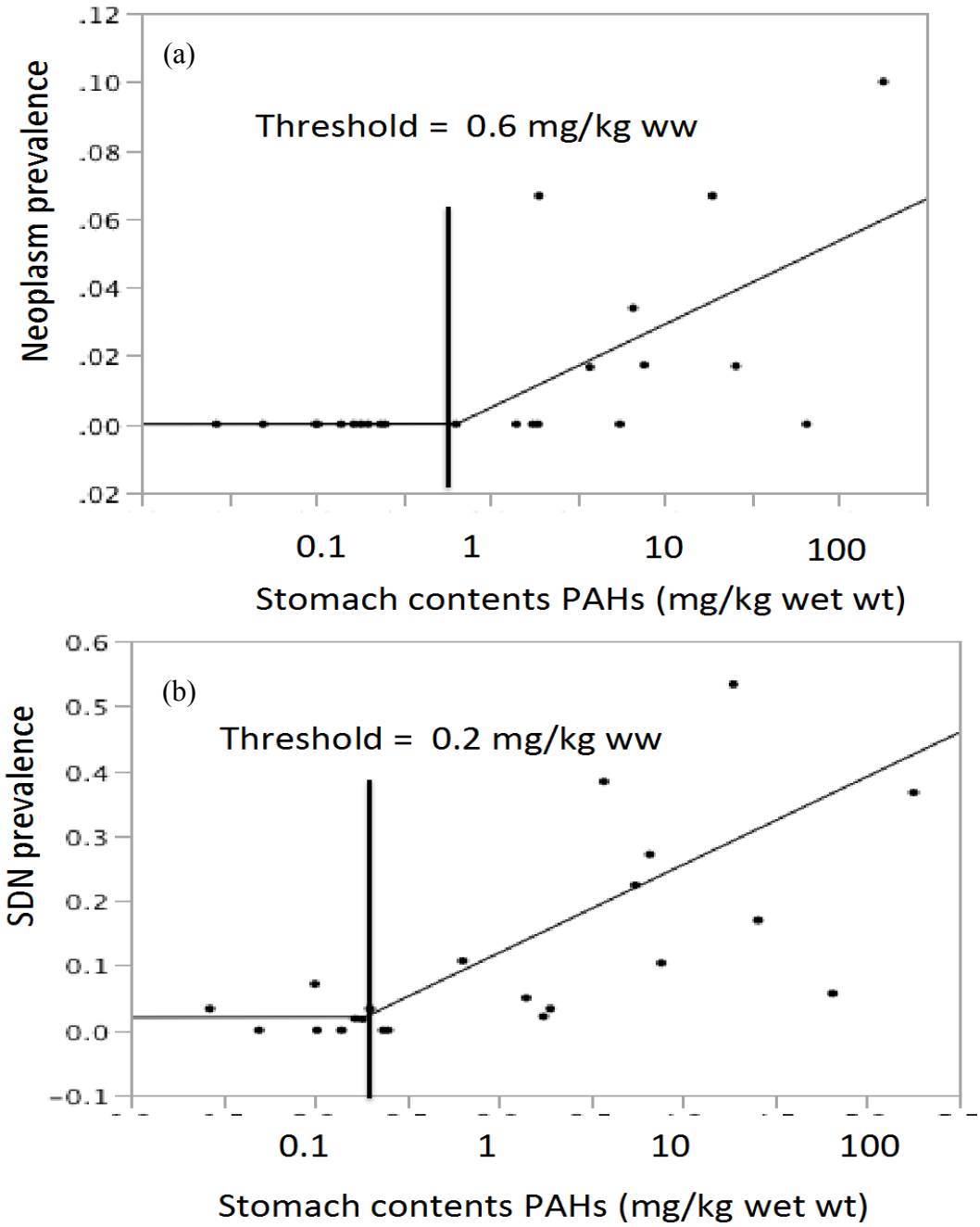


Figure 1. Hockey stick regression of lesion prevalence in English sole versus total polycyclic aromatic hydrocarbons (PAHs) in stomach contents (mg/kg ww) for a) neoplasms and b) specific degeneration/necrosis (SDN). Vertical lines indicate threshold concentrations.

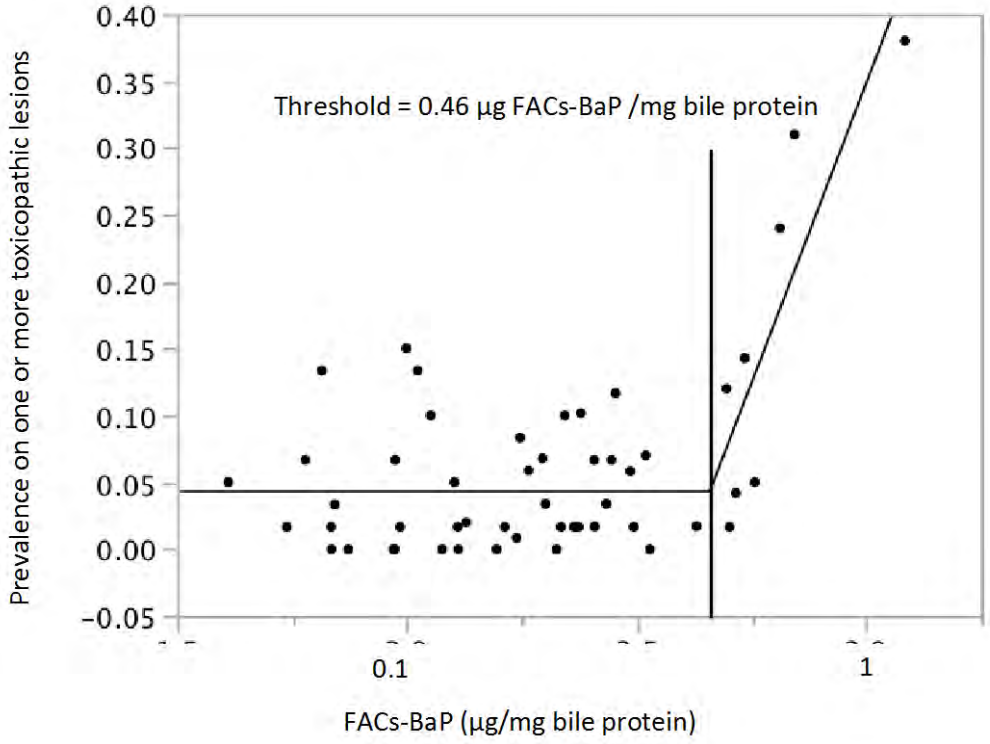


Figure 2. Hockey stick regression of lesion prevalence in English sole versus total polycyclic aromatic hydrocarbon (PAH) metabolites in bile fluorescing at BaP wavelengths (µg/mg bile protein one or more toxicopathic lesions. Vertical line indicates threshold concentration.

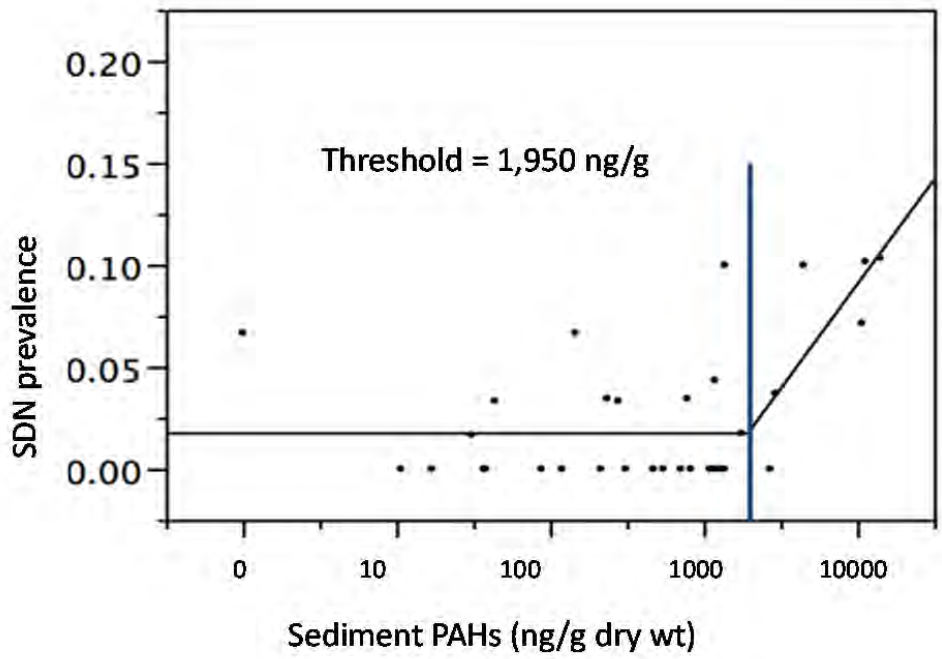
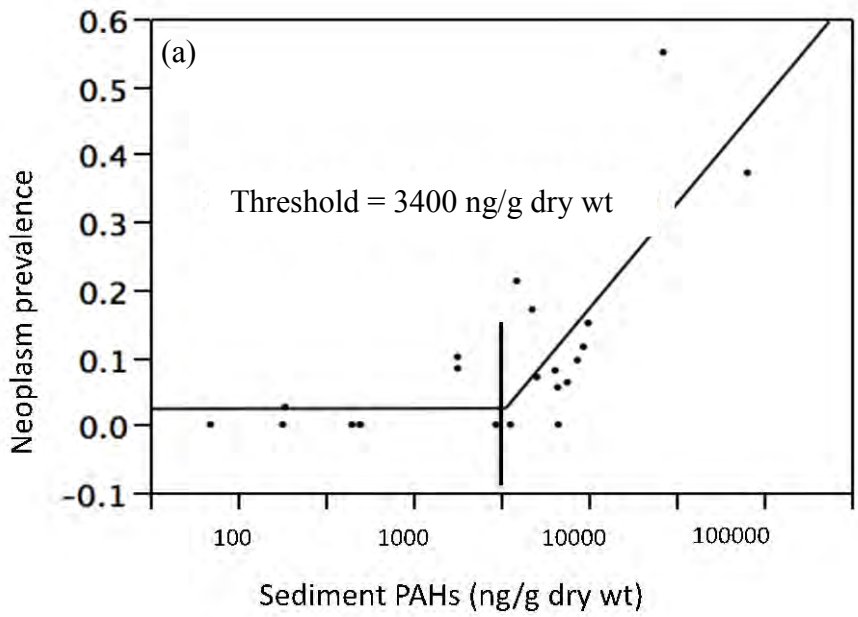


Figure 3. Hockey stick regression of specific degeneration/necrosis (SDN) prevalence vs. sediment total polycyclic aromatic hydrocarbon (PAH) concentration (ng/g dw) in starry flounder. Data were collected as part of NOAA's NBSP for the U.S. northwest coast (Myers et al. 1994). Vertical line indicates threshold concentration.



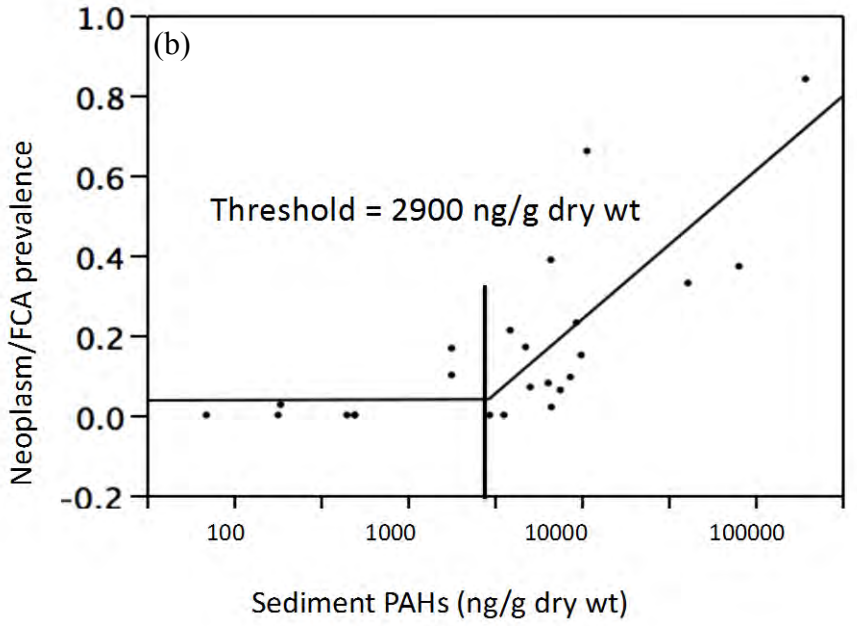


Figure 4. Hockey stick regression of (a) neoplasm prevalence vs. sediment total polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g dw) and (b) neoplasm and foci of cellular alteration (FCA) prevalence for brown bullhead. The threshold estimate for neoplasms is 3,400 ng/g dw total PAHs, while the threshold estimate for neoplasms and FCA is 2,900 ng/g dw total PAHs. Vertical lines indicate threshold concentrations.

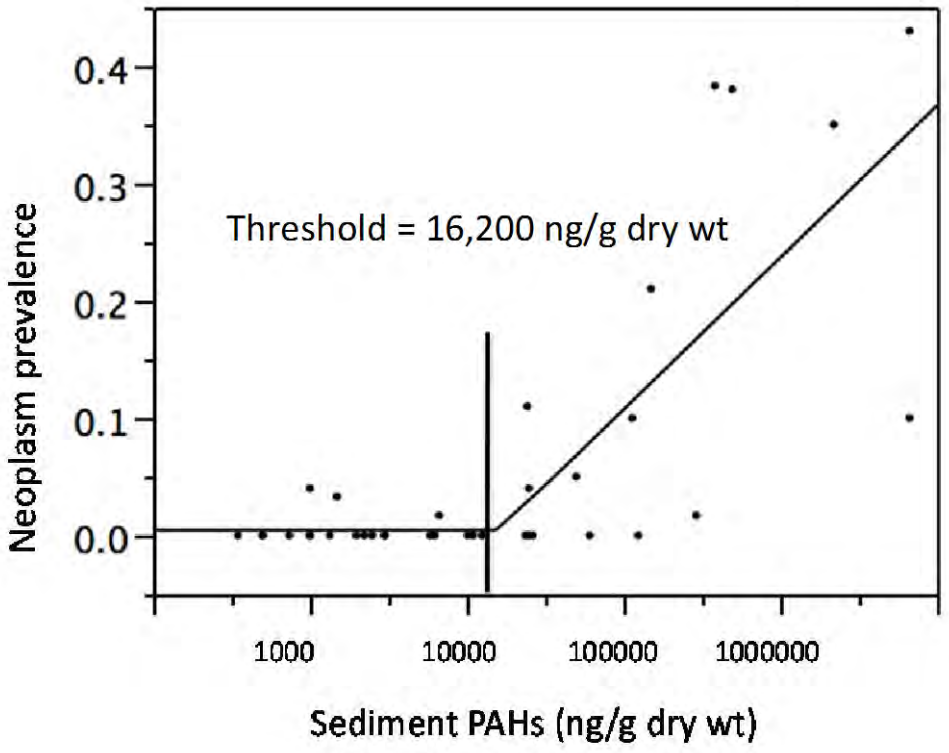


Figure 5. Hockey stick regression of neoplasm prevalence vs. sediment total polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g dw) for killifish. The threshold value for this regression is 16,200 ng/g dry wt total PAHs (represented by vertical line).

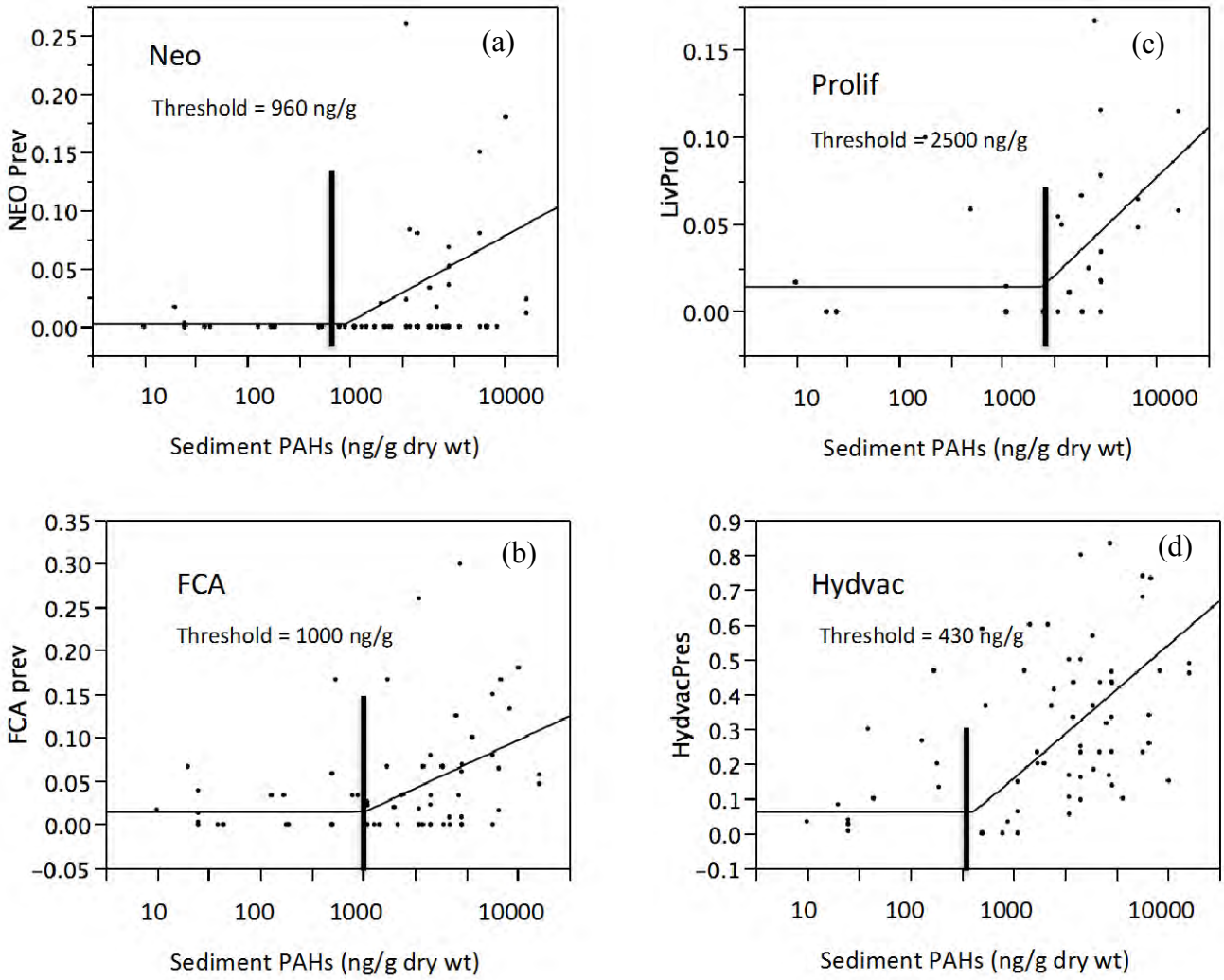


Figure 6. Hockey stick regression of hepatic lesion prevalence in winter flounder versus total polycyclic aromatic hydrocarbon (PAH) concentrations (ng/g dry wt) for a) neoplasms (Neo); b) foci of cellular alteration (FCA); c) proliferative lesions (Prolif); and d) hydropic vacuolation (Hydvac). Vertical lines represent threshold concentrations.

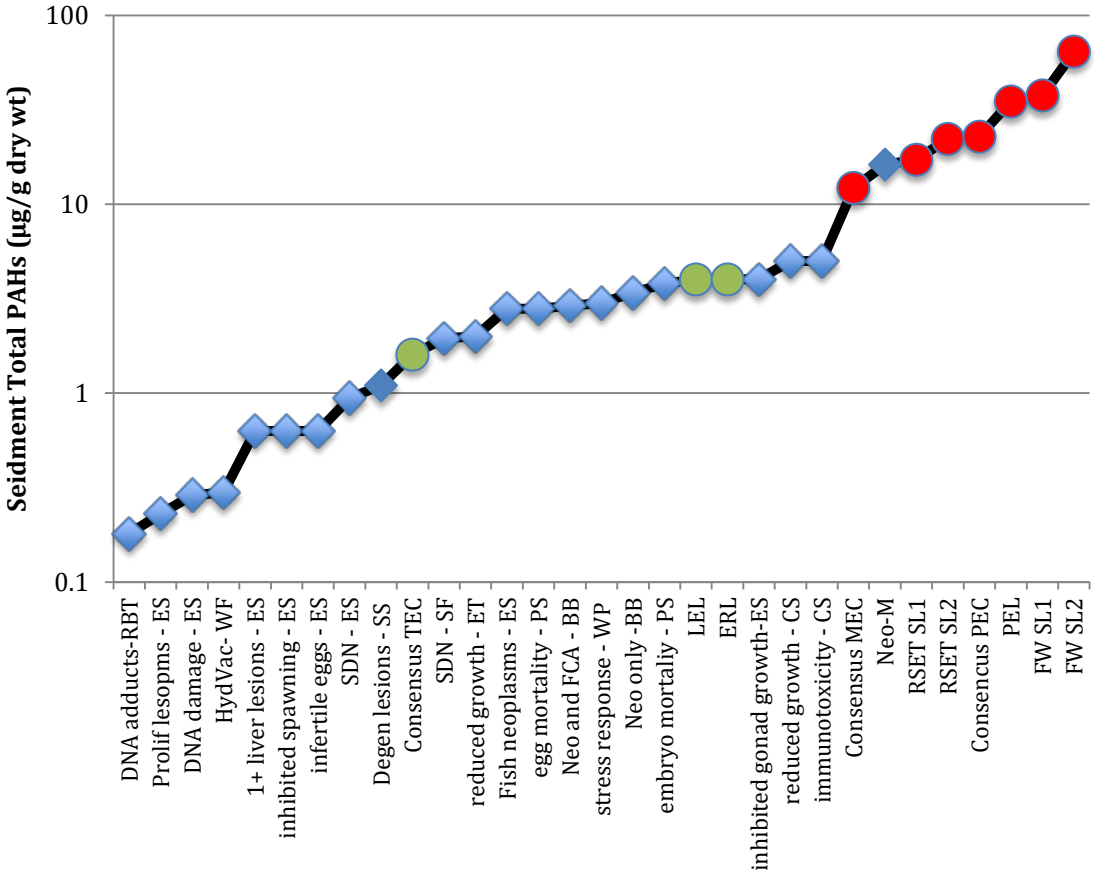


Figure 7. Sediment total polycyclic aromatic hydrocarbon (PAH) concentrations in µg/g or ppm (multiply by 1,000 to convert to ng/g or ppb) vs. biological effects in fish (blue diamonds), as compared with commonly used no-to-low effect (green circles) and probable-effect (red circles) sediment quality guidelines. RBT = rainbow trout; ES = English sole; WF = winter flounder; SS = Senegalese sole; SF = starry flounder; PS = pink salmon; BB = brown bullhead; WP = white perch; CS = Chinook salmon; M = mummichog. TEC = threshold effect concentration (Macdonald et al. 2000); LEL = low effect level; ERL = effect range low (Long et al. 1998); PEC = probable effect concentration (MacDonald et al. 2000); PEL = probable effect level (MacDonald et al. 2000).

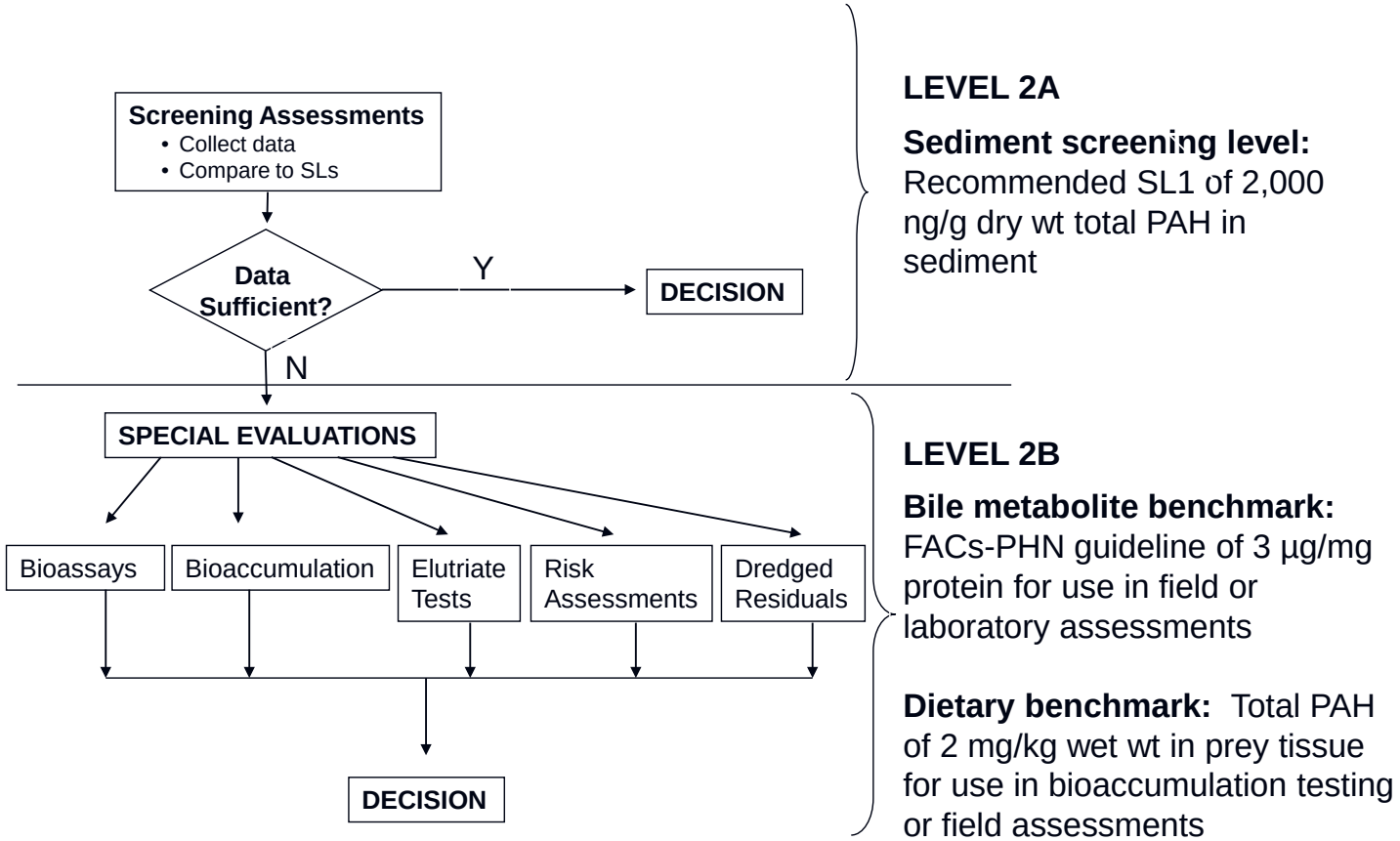


Figure 8. Modified sediment evaluation framework for impacts of total polycyclic aromatic hydrocarbons (PAHs) in fish.

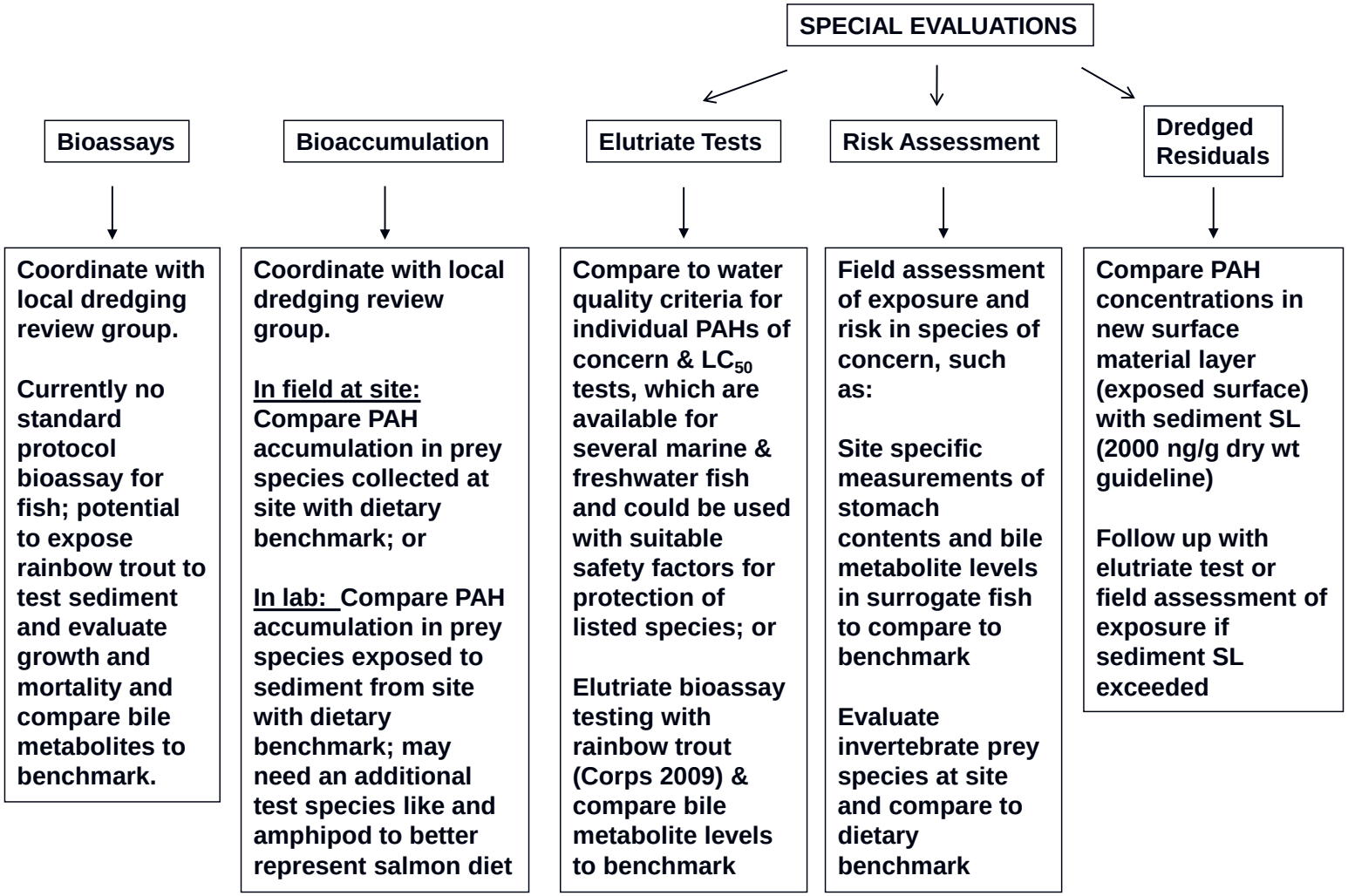


Figure 9. Potential options for screening level 2 test and special evaluations for sediments that fail sediment screening guidelines for total polycyclic aromatic hydrocarbons (PAHs).

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