National Oceanic and Atmospheric Administration

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MEMORANDUM FOR: Rachel Friedman, F/NWO5
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FROM: Tracy Collier, F/NWC5,
Ecotoxicology Program Manager

THROUGH: John Stein, F/NWC5,
Division Director

SUBJECT: Submission of 'white paper'. An analysis in support sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish.

Enclosed is our report to your office concerning a technical analysis of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish. A previous version of this document was peer reviewed and we have incorporated several changes in response to comments received. Ms. Lyndal Johnson is the senior author of this report. Please contact any of us with questions you may have concerning the contents of this report.

An analysis in support of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish

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Background

Polycyclic aromatic hydrocarbons (PAHs) are common contaminants in sediments of Puget Sound and other industrialized embayments worldwide. The majority of PAHs associated with sediments in Puget Sound as well as at other coastal urban sites originate from petroleum and combustion products (Varanasi et al. 1992, MacDonald and Crecelius 1994). Sources include industrial discharges, creosote from treated wood, municipal runoff, and atmospheric emissions from incineration and automobile emissions. PAHs are also introduced into marine systems through accidental spills of fuel oil, crude oil, and other petroleum products, and from non-point sources.

PAHs, particularly the higher molecular weight compounds, tend to adsorb to organic or inorganic matter in sediments, where they can be trapped in long-term reservoirs. Although only a portion of sediment-adsorbed PAHs are readily bioavailable to marine organisms, there is substantial uptake of these compounds by resident benthic fish through the diet, through the water column, and through exposure to sediment. Benthic invertebrate prey are a particularly important source of PAH exposure for marine fishes, as PAHs are bioaccumulated in many invertebrate species (Varanasi et al. 1989, 1992; Meador et al. 1995).

A notable feature of PAHs is that they are metabolized extensively in vertebrates, including fishes, unlike many chlorinated hydrocarbons, which bioaccumulate in tissues. Cellular metabolism of PAHs (Varanasi et al. 1989) results in conversion of these hydrophobic compounds into polar, water soluble forms that can be readily excreted from the organism. Consequently, parent PAHs generally do not bioaccumulate in fish or other vertebrates, although metabolites present in food are bioavailable to the consumer (McElroy et al. 1991, James et al. 1991) and PAH-DNA adducts accumulate in the liver of fish chronically exposed to sediment associated PAHs (Reichert et al. 1998). Moreover, PAHs are capable of causing a variety of deleterious effects in exposed animals. While metabolism serves mainly as a pathway of detoxication for PAHs, some of the metabolites that are intermediates in this process possess carcinogenic, mutagenic and cytotoxic activity. Laboratory tests with rodents, as well as epidemiological studies, have established that certain

PAHs, as well as some of their metabolites, are chemical carcinogens. These compounds are also known to be immunotoxic and to have adverse effects on reproduction and development (Klaasen 1997). Over the past 20 years, a number of studies have been conducted on effects of PAHs on marine fish and other marine biota. These studies show that PAHs exhibit many of the same toxic effects in fish as they do in mammals. For example, liver cancer and related lesions have been documented in several species of wild fish as a result of environmental exposure to PAHs (Moore and Myers 1994).

Of the fish in Puget Sound, English sole (*Pleuronectes vetulus*) is one of the most extensively studied species in pollution monitoring programs. Several features make it suitable as a sentinel species for studies of the effects of environmental pollution. It is widely distributed along the Pacific coast of the USA, in both urban and non-urban environments. Because of its benthic life history as a juvenile and as an adult, it is particularly likely to take up sediment-associated contaminants, both through direct contact with sediments and through the diet. In addition, with the exception of a winter spawning migration, English sole within Puget Sound are relatively sedentary, and show high fidelity to the sites where they are resident (Day 1976). Consequently, biological effects observed in English sole are generally an accurate reflection of PAH exposure at the sites where they are collected. A large body of data has been generated on the effects of PAHs on this species, in both field and laboratory studies. These data show that English sole from PAH-contaminated embayments are highly susceptible to the development of liver cancer and related lesions (Myers et al. 1994, 1998a), and also appear to be prone to a number of other adverse health effects, including reproductive abnormalities, immune dysfunction, and alterations in growth and development (Arkoosh et al. 1996; Johnson et al. 1998).

Liver disease, including cancer, is one of the most dramatic and best-documented effects of PAH contamination on English sole in Puget Sound (Myers et al. 1987, 1990, 1994, 1998a,b; O'Neill et al. 1999; PSWQAT 2000) and other embayments along the west coast (Myers et al. 1994, 1998a). In general, the prevalence of liver disease increases with increasing urbanization (Myers et al.

1998a; O'Neill et al. 1999). Typically, between 25 and 40% of adult English sole sampled from urban embayments, such as Elliott Bay and Commencement Bay, exhibit neoplastic, preneoplastic, or unique degenerative liver lesions, as compared to 3 to 8% of adult sole from nonurban and moderately urbanized sites. In several independent statistical analyses of field data from different studies (Rhodes et al. 1987, Landahl et al. 1990, Myers et al. 1990, 1994, 1998a,b) exposure to PAHs was identified as the major risk factor for neoplasms and related liver lesions in English sole. A cause-and-effect relationship between PAHs and toxicopathic liver lesions in English sole is supported by induction of degenerative, proliferative, and preneoplastic lesions, identical to those observed in field-collected fish, when sole are exposed in the laboratory to model carcinogenic PAHs such as benzo[a]pyrene (BaP) and to extracts of sediments from PAH contaminated sites (e.g., Eagle Harbor) in Puget Sound (Schiewe et al. 1991) where lesions are observed.

In addition to toxicopathic liver disease, English sole residing in contaminated areas in Puget Sound also suffer from various types of reproductive impairment. Field studies show that female English sole from areas with high concentrations of PAHs in sediment are less likely to enter vitellogenesis and have lower plasma concentrations of the female reproductive hormone, 17β-estradiol, than sole with low levels of contaminant exposure (Johnson et al. 1988, 1997, 1999). At minimally contaminated sites within Puget Sound (sediment total PAH concentrations < 500 ppb dry wt), approximately 80-90% of adult females undergo gonadal development, while at more highly contaminated sites (e.g., the Duwamish and Hylebos Waterways and Eagle Harbor) the percentage declines to 40-60%. In statistical analyses of these data, exposure to PAHs emerges as a major risk factor for inhibited ovarian development in adult sole. English sole from PAH-contaminated areas also display increased ovarian atresia and reduced egg production (Johnson et al. 1988, 1997). Results of these field studies are supported by laboratory experiments showing that pretreatment of gravid female English sole with extracts of contaminated sediment or crude oil containing high levels of PAHs decreased levels of endogenous estradiol (Stein et al. 1991; Johnson et al. 1995). More recent experiments suggest that exposure to benzo[a]pyrene or PAH-contaminated sediment may suppress estradiol-induced vitellogenin production in English sole (Anulacion et al. 1997).

Studies also suggest that English sole from contaminated areas that do successfully enter vitellogenesis may experience inhibited spawning ability and reduced viability of eggs and larvae. When gravid English sole from four Puget Sound sites (Port Susan, Sinclair Inlet, the Duwamish Waterway, and Eagle Harbor) were brought into the laboratory and artificially induced to spawn, spawning success was significantly lower in fish from the two most contaminated sites, Eagle Harbor and the Duwamish Waterway (Casillas et al. 1991). Moreover, exposure to PAHs in the water column (e.g., fluoranthene at 0.075 to 7.5 mg of PAH/liter of seawater) caused larvae to become disoriented and to exhibit signs or narcosis, with mortality at the higher concentrations (Eddy et al. 1993).

Effects of PAHs on reproduction have not been studied as extensively in male as in female English sole. There is evidence, however, that males may also be susceptible to PAH-related reproductive dysfunction. Preliminary studies suggest that although testicular development in male sole from PAH contaminated sites is relatively normal, plasma concentrations of 11-ketotestosterone and testosterone are reduced in fish with particularly high concentrations of PAH metabolites in bile, a measure of PAH exposure (Sol et al. 1999).

English sole growth also appears to be affected by exposure to PAHs. Recent laboratory studies (Kubin 1997; Rice et al. 1999) show reduced growth in juvenile English sole exposed to PAHs through contaminated sediment or diet. These effects have not yet been corroborated in wild populations of English sole, but if they do occur, they could impact sole populations by reducing fecundity or altering the time to sexual maturity (Brandt et al. 1992). Slow growth rates have also been associated with increased juvenile mortality in several fish species (Peterson and Wroblewski 1984; McGurk 1996; Lorenzen 1996).

Analysis

The purpose of the following analysis was to use existing data on PAH effects in English sole to determine sediment PAH concentrations at which biological injury is likely to occur. We selected English sole as the target species because of its sensitivity to PAHs, its presence at sites within Puget Sound where resource damage assessment and restoration efforts are planned or underway, and because there is substantial information on health effects of PAHs in this species from field studies, which have been corroborated, in large part, by laboratory studies. Our initial focus was on liver lesions in English sole, because of the abundance of data available for this health effect, and the preponderance of evidence for a cause and effect relationship between PAHs and the development of liver cancer in English sole. However, we also include data on DNA damage, reproductive effects and impaired growth.

For this analysis, biological effects were linked directly to sediment PAH concentrations at sites where test animals were resident, rather than to contaminant body burdens. This was done in primarily because PAHs are metabolized in fish and do not bioaccumulate (Varanasi et al. 1989), but also because the large volume of field data available on site-specific sediment PAH concentrations and associated biological effects in English sole made such an analysis feasible. Additionally, the sediment-based analysis is more directly applicable to the development of appropriate sediment quality guidelines for the protection of estuarine fish. An advantage of this method is that it is based on health effects measured in a native organism collected from its natural environment. As such, there are fewer questions on the environmental relevance of this approach, compared to laboratory bioassays. The effects considered reflect long-term exposure, potentially over the life of the organism, and incorporate exposure through all routes of uptake, including diet, water, and directly from sediment through skin contact or ingestion. In laboratory exposure studies, exposure generally lasts for only a limited period of time, and uptake is typically restricted to a single route of exposure, such as diet, or direct contact with sediments. A disadvantage of the field-based approach is that it may not adequately account for biological effects resulting from exposure

to other contaminants or contaminant mixtures present in sediments. However, this can be mitigated to some extent by choosing endpoints, such as liver cancer and related lesions, for which PAHs are known to be a strong causative factor.

Liver disease

Using data collected over the past 10 years from a variety of field studies in Puget Sound and on the West Coast (Myers et al. 1998a; Brown et al. 1998) we statistically determined threshold PAH concentrations at which liver lesion prevalences begin to increase. The hockey stick regression model (Horness et al. 1998) was employed in these analyses. Hockey stick regression is one of a number of standard dose-response models (Gad and Weil 1991), and has been used in a variety of epidemiological and toxicological studies (e.g., Cox et al. 1989; Hammer et al. 1974; Gordon and Fogelson 1993). The model consists of two linear segments whose "blade and handle" shape resembles a hockey stick (Yanagimoto and Yamamoto 1979). In the present application (Fig. 1), the lower segment was assigned a slope of zero to represent a constant low-level background effect. The upper segment was defined as a linear function with a positive slope. The upper segment represents a dose-response relationship above a threshold that is estimated by the point of intersection of the two segments, as follows:

Effect = background for $SC < SC_T$

Effect = background + $\beta(SC-SC_T)$ for $SC > SC_T$

SC = sediment contaminant concentration, SC_T = threshold sediment contaminant concentration[1],

SC_T(threshold contaminant concentration), background (the spontaneous background effect) and β (the slope of the curve) are estimated parameters. Effect and SC (sediment contaminant concentration) are the dependent and independent variables.

[1] The notation SC_T (threshold sediment contaminant concentration) is used in this paper to be consistent with Horness et al. 1998, but in application this is equivalent to SET (sediment effect concentration) used in the white papers by Meador.

We realize that the risk analysis models used in epidemiology typically assume that cancer induction is a non-threshold phenomenon, and our choice of a threshold model for this exercise is not meant to imply that a true threshold exists in the process of carcinogenesis in English sole. Rather, the model was chosen for pragmatic reasons, to facilitate our identification of exposure levels at which statistically detectable and biologically relevant increases in lesion prevalence would be expected to occur in wild fish populations. The application of a threshold model is supported by the fact that, for most carcinogens, repair processes and compensatory mechanisms exist that can counteract the effects of carcinogens at low levels of exposure, even though one molecule of a carcinogen could theoretically induce an initiated cell, leaving no latitude for a threshold. Based on a similar rationale, the application of a threshold approach for regulating exposure to some carcinogens has been suggested for human health risk management (Lutz 1998; Gaylor et al. 1999; Butterworth and Bogdanffy 1999).

The model was used to relate sediment PAH concentrations to prevalences of the four most common toxicopathic hepatic lesion types found in English sole. These four types were 1) neoplasms; 2) preneoplastic foci of cellular alteration (FCA), which are thought to be precursors of neoplasms; 3) specific degeneration/necrosis (SDN), a degenerative lesion associated with exposure to PAHs; and 4) non-neoplastic proliferative lesions, such as hyperlasia of hepatocytes or bile ducts. Additionally, a composite category was examined consisting of the presence of one or more of any of the first three lesions (neoplasms, FCA, and SDN) in an individual fish. Lesion prevalences were based on examination of between 30 and 60 fish per site. Collection site locations are described in detail in Horness et al. (1998) and Myers et al. (1994). Because age is a significant risk factor for preneoplastic and neoplastic lesions in English sole, all fish less than two years of age were excluded from prevalence calculations. This exclusion is based on data indicating that fish below this age have not had sufficient time to develop these types of hepatic lesions even at high exposure levels (Myers et al. 1998b).

To characterize the level of contamination present at a given site, surficial sediment samples were collected from the area in which the fish were captured and analyzed for toxic contaminants (Sloan et al. 1993) including both low and high molecular weight PAHs (Table 1). The correlation between the low and high molecular weight analytes was too high to consider them as separate factors ($r^2 = 0.94$). Moreover, there is only limited direct information to link a specific subset of PAHs to the effects that have been observed. Consequently, total sediment PAH content (the sum of all analytes shown in Table 1) was used as the contaminant category of interest for this analysis. It should be noted that the sediment samples used in this study contained not only lower molecular weight compounds associated with oil and other petroleum products, but significant concentrations of higher molecular weight PAHs which are typical of industrial urban sites (Brown et al. 1998). Consequently, sediment PAH effects thresholds generated with these data may not be fully applicable at sites where sediments contain primarily low molecular weight PAHs, whose carcinogenic potency is generally not as great as that of high molecular weight PAHs (NTP 1998).

As chemical concentration data are generally log-normally distributed, the PAH concentrations were log-transformed prior to the regression. Thus, log (sediment PAH concentration) was used for the independent variable SC. Lesion prevalence was used for the dependent variable (Effect). The model was fitted using a nonlinear regression parameter estimation routine using the SAS statistical package JMP. Confidence intervals were computed at an α of 0.1, rather than the standard 0.05, to increase the statistical power to detect a threshold and decrease the likelihood of a type II error (false negatives) without greatly increasing the likelihood of type I errors (false positives) (Peterman 1990).

Depending on the specific type of lesion, threshold effect estimates ranged from 54 to 2800 ppb (ng/g dry wt) (Fig. 2a-d; see Table 2 for regression parameters). All threshold estimates were statistically significant ($\alpha = 0.1$) except for that for FCA, for which no lower confidence bound was found. At 2,800 ppb (90% CI = 11-5500 ppb), neoplasms exhibited the highest PAH threshold. FCA exhibited the lowest threshold (54 ppb; CI = 0-870 ppb) and the lack of a lower confidence

limit suggests that FCA prevalence in English sole may be directly proportional to sediment PAH content over the entire range of contaminant concentration rather than operating through a threshold relationship. The calculated threshold concentration for SDN, the most common toxicopathic lesion in English sole, was 940 ppb (CI = 600-1400 ppb).

DNA damage

The threshold analysis for DNA damage was done in the same manner as the analyses for liver lesions, but using the mean concentration of aromatic compound-DNA adducts in liver of English sole from the sampling sites as the outcome variable (i.e., Effect). As with liver lesions, our application of a threshold model for this analysis does not imply that DNA adduct formation itself is a threshold phenomenon. Our objective was to use to the model to assist us in identifying the PAH exposure level where increases in mean DNA adduct levels in liver could first be detected in English sole populations. Mean DNA adduct levels were based on analyses of liver tissue from 3-10 fish per site. The threshold effect estimate was 290 ppb (ng/g dry wt), with a 90% CI of 6-1380 ppb (Fig. 3; see Table 2 for regression parameters). This threshold estimate is at the lower end of the range of threshold estimates for liver lesions, which is toxicologically consistent with the hypothesis that this type of DNA damage is a precursor to more overt pathological conditions in the liver, and a necessary step in the development of certain types of neoplasms (Farber and Sarma 1987). A threshold in this range is also supported by a laboratory study (French et al. 1996) in which exposure to sediments contaminated with 1200 ppb dry wt PAH resulted in DNA adduct concentrations in English sole liver of 15-20 adducts/mol nucleotides, in comparison with 5-6 adducts/mol nucleotides in fish exposed to sediments containing 20 ng/g PAH. The threshold model predicts adduct levels of 28 adducts/mol nucleotides at 1200 ng/g dry wt PAH, and 5 adducts/mol nucleotides and 5 ng/g dry wt PAH. The laboratory study, however, did not include any exposure treatments between 20 ng/g and 1200 ng/g PAH. Additional data on DNA adduct levels in sole exposed to sediments with PAH concentrations in the 100-1000 ppb range would be necessary to provide laboratory validation of the threshold estimate.

DNA adducts represent a tissue-level alteration or injury that is caused by PAH exposure, are correlated with other health effects such as liver disease, and can develop with relatively short-term exposure of days to weeks (Reichert et al. 1998). In addition, hepatic DNA adducts in fish are persistent and accumulate with chronic exposure to sediment-associated PAHs (French et al. 1996, Reichert et al. 1998). As such, DNA adducts can be used as an indicator of PAH exposure and effects for estimating the likelihood of PAH-induced injury in species that are short-term residents of urban estuaries, such as anadromous salmonids.

Reproductive dysfunction

Although we do not have sufficient data to statistically determine precise thresholds for other effects in English sole, available information indicates that additional types of impairment begin to occur at sediment PAH concentration in a similar range. For example, in Figure 3, prevalences of several types of reproductive impairment observed in female English sole (Johnson et al. 1988, 1999; Casillas et al. 1991) are plotted against sediment PAH concentrations at the sites where fish were collected in Puget Sound. All fish utilized in these analyses were of reproductive size and age, at least 3 years of age or 30 cm, which is when female English sole typically reach maturity (Garrison and Miller 1982; Johnson et al. 1991). The plots indicate that spawning failure and egg infertility begin to increase above background levels at sediment PAH concentrations between 250 and 1600 ppb, while the proportion of sole failing to undergo ovarian maturation begins to increase at concentrations between 1600 and 10,000 ppb. If we estimate the effect thresholds by taking the geometric mean of these two points, this yields an effect threshold of 4000 ppb for inhibition of ovarian development, and a threshold of 630 ppb for all other reproductive endpoints.

Exposure-response relationships for reproductive endpoints at sediment PAH concentrations above the threshold levels were estimated using the formula for fitting the upper segment of the hockey-stick regression:

Effect = background + $\beta(SC - SC_T)$

Estimated threshold and background effect levels were substituted into the model, rather than calculated through regression analysis as for liver lesions and DNA adducts. Background effect levels were assumed to be identical to those found at reference sites with the lowest sediment PAH concentrations, so that background = 0.15 for proportion failing to mature; 0.12 for proportion failing to spawn; 0.25 for proportion of abnormal larvae; and 0.38 for proportion of infertile eggs. The estimated SC_Ts used for this analysis were 4000 ppb for failing to mature and 630 ppb for all other endpoints, as described above. Parameter estimates derived from the regression analysis are shown in Table 2, and plotted regression curves are shown in Fig. 3. The horizontal portion of the curve represents background values for these endpoints, estimated from field data, while the upper portion is the fitted hockey stick regression model.

The results show that the proportion of sole that failed to mature, the proportion that failed to spawn and the proportion of eggs spawned that were infertile were all significantly correlated with increasing sediment PAH concentration, as indicated by positive estimates for β with confidence intervals that excluded zero. However, the proportion of abnormal larvae produced showed no clear relationship with sediment PAH level; the confidence interval for β included zero, indicating that the slope of the line describing the relationship between sediment PAH concentration and the proportion of abnormal larvae was not statistically different from zero. Consequently, this endpoint was excluded from further analyses of PAH effect thresholds and exposure-response relationships.

While more data would be needed to calculate SC_Ts and CIs for the reproductive endpoints, this analysis currently provides the best available threshold estimates to compare to the SC_Ts for liver lesions in English sole. The results also suggest that these types of reproductive impairment are found at sediment PAH concentrations similar to those associated with the development of hepatic lesions.

Growth reduction

Data from two recent laboratory exposure studies suggest that declines in growth of juvenile English sole also occur from exposure to PAHs (Kubin 1997; Rice et al. 1999). Although neither of these experiments were 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) exposed juvenile English sole to sediments contaminated with PAHs at concentrations of approximately 4000 ppb dry wt, 2000 ppb dry wt, and < 50 ppb dry wt for six months[2]. For the first three months, growth rates were similar for all treatments (1.0-1.1% per day for weight, and 0.36-0.38% per day for length), but during the next three months, growth rates were significantly lower in the high exposure group. The percent change in weight was 0.35% per day fish exposed to the highest concentration of PAHs, as compared to 0.43% in control fish, while the percent change in length was 0.13% per day in exposed fish as compared to 0.16% per day in control fish. The fish exposed to sediments with moderate PAH concentrations showed no significant decrease in growth rate relative to control fish. These data suggest a threshold for growth effects in the 2000-4000 ppb range. Actual threshold effect concentrations could be lower, as in this experiment, uptake of PAHs was from sediment and water only, and in the natural environment, substantial exposure would also occur through the diet, with ingestion of invertebrate prey species residing in contaminated sediment.

A study by Rice et al. (1999) confirmed both the effect of PAHs on growth of juvenile English sole and the importance of dietary exposure. The findings showed significantly reduced weight in juvenile English sole fed polychaete worms reared on sediments containing 3000-4000 ppb dry wt of PAHs, after an exposure period of only 28 days. The percent change in weight was markedly less (0.05-0.1% per day) in exposed fish, as compared to control fish (1.1-1.2% per day). If sole

[2] Sediment PAH concentrations for this study were determined using the HPLC/PDA screening system of Krahn et al. (1991), which provides an estimate of PAH concentration by measurement of fluorescent aromatic compounds (FACs) in sediments. Total PAH concentrations, as determined by GC/MS, were estimated based data from on (Krahn et. al. 1988), in which parallel sediment samples from a variety of urban and non-urban sites along the U.S. West Coast were analyzed using both methods.

had been exposed to PAHs through uptake from both sediment and diet, as they are in the natural environment, and for a more extended period, it is reasonable to assume that effects on growth could be observed at lower sediment PAH levels, more comparable to the SC_T for liver lesions. If, for example, a safety factor (Mount 1977) of 2 were applied to the 3000 ppb sediment PAH level associated with growth reductions in Rice et al. (1999), to account for additional PAH uptake through sediment and the water column, this would yield a threshold concentration of 1500 ppb, which is comparable to threshold concentrations associated with liver lesions, DNA damage, and certain reproductive effects.

The central finding from these data is that English sole exposed to sediment concentrations where toxicopathic lesions are observed are also likely to experience negative impacts on growth and reproduction. All three types of effects represent significant injury to the health of affected fish. The impacts on growth and reproduction are particularly likely to affect the productivity of stocks from contaminated sites because of their potential to reduce fecundity and age of sexual maturation.

Sources of Uncertainty in Threshold Estimates

Although we believe that the present analysis provides good guidance on approximate sediment PAH concentrations associated with injury in English sole, several variables that could influence exposure-response relationships cannot fully accounted for in this analysis of current data. Among the more important factors are fish age, length of exposure, and exposure to other contaminants.

- The present analysis adjusts for fish age only in a very basic manner. Very young fish were excluded, as well as sites where the majority of fish collected were subadults. Because the analysis was done on a site basis rather than an individual fish basis, it was not possible to fully adjust for the increasing risk of disease development with age. Moreover, the number of animals lost to disease is not known, and is not incorporated into the analysis. Because of these limitations, the analysis may underestimate the lifetime cancer risk to English sole of a particular PAH exposure concentration.

- The co-occurrence of other contaminants along with PAHs at sites where English sole were collected is not incorporated into the analysis. While the correlations between PAH exposure and the endpoints we measured in this study are statistically valid and well-supported by other scientific evidence, sediments at the sites included in the analysis contain a variety of other compounds (e.g., PCBs, chlorinated pesticides, and heavy metals; see Brown et al. 1989 and Meador et al. 1994 for more detailed information) that are promotors of carcinogenesis, or reproductive toxicants. These compounds could act either additively or synergistically with PAHs to produce the observed health impacts. Their presence could alter disease prevalence, and they are likely an important factor contributing to variability in response among fish populations at different sampling sites. Incorporating the effects of co-occurring chemicals into PAH threshold estimates is beyond the scope of the present analysis. The potential of interactive effects among co-occurring compounds is an area that warrants additional research because it does introduce uncertainty in the sediment threshold values.

- The suite of PAHs used to calculate total PAH concentrations for the development of effects thresholds in this analysis differs somewhat from the group of 16 priority PAHs recommended by EPA (U.S. EPA 1986). The EPA priority PAHs include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, and 2-methylnaphthalene as LAHs, and fluoranthene, pyrene, benzo(a)anthacene, chrysene, benzofluoranthene, benzo(a)pyrene, ideno(1,2,3-c,d)pyrene, dibenzo(a,h)anthracene, and benzo(g,h)perylene as HAHs. Recalculation of the thresholds with this alternate group of PAHs could yield different threshold estimates. However, preliminary analyses of sediment PAH thresholds for liver lesions in English sole have been performed using data from Washington State's Puget Sound Ambient Monitoring Program, in which total PAHs are calculated based on EPA's set of 16 compounds. Liver lesion thresholds ranged from 500-2000 ppb dry wt total PAH in sediment (O'Neill et al. 1999). These thresholds are very similar to those presented in this paper, which were derived from our NBSP data set (Myers et al. 1998a; Brown et al. 1998). These results

suggest that the thresholds we have determined are not highly dependent on the exact suite of individual PAHs used to calculate total PAHs. On the other hand, the applicability of these thresholds to estuarine environments where the suite of PAHs present differs substantially from those typically present at industrialized urban sites is not known. Additional analysis with a variety of datasets would be needed to better established the applicability of these threshold estimates in other estuarine environments.

The present analysis establishes PAH sediment thresholds for protection of a single species, English sole. Their application to other estuarine fish is not known. Species differences in sensitivity to the effects of PAHs are well-documented (Myers et al. 1998a; Johnson et al. 1992, 1998a; Collier et al. 1992, 1993; Anulacion et al. 1998), so variation in PAH effects thresholds would be expected. However, previous research with English sole suggests it is relatively sensitive to PAH-associated injury (Anulacion et al. 1998; Collier et al. 1992; Johnson et al. 1998b; Myers et al. 1998a), so sediment thresholds developed for this species may be protective of other fish as well. We have conducted limited preliminary analyses with other bottomfish species, including white croaker and winter flounder (Lomax et al. 1994), which suggest that sediment PAH thresholds for some categories of non-neoplastic proliferative and degenerative lesions (e.g., SDN) are similar to those for English sole. However, a more thorough investigation of species differences is needed.

Sediment Quality Threshold Guidance

In order to assess the likely degree of injury experienced by English sole exposed to sediments with different sediment PAH concentrations, expected prevalences of liver lesions and reproductive abnormalities were calculated using the regression equations from Table 2 for total PAH concentrations ranging from 50-100,000 ppb (ng/dry wt). As illustrated in Table 3, liver lesion prevalences, as well as levels of other detrimental effects in English sole, were generally close to levels characteristic of fish from uncontaminated sites at sediment PAH concentrations below 1000 ppb. This concentration approximates the upper confidence levels of the thresholds for DNA

damage and the occurrence of one or more hepatic lesions, and is close to the estimated threshold of 630 ppb for several reproductive effects. Above 1000 ppb, increases are observed in several categories of liver lesions, and reductions are observed in spawning ability and egg quality. On this weight of evidence basis, the sediment PAH threshold below which no significant carcinogenic or adverse reproductive effects in English sole are observed is estimated to be 1000 ppb (ng/g dry wt), or approximately 50 mg/kg total organic carbon (TOC), assuming a sediment TOC content of 2%, a fairly typical value for Puget Sound sediment (Michelsen and Bragdon-Cook 1993). With sediment PAH concentrations at or below this level, sole should exhibit only minimal liver injury, and little or no disruption of growth or reproductive function, and relatively low levels of DNA adducts. While this threshold is well below current sediment quality criteria for PAHs as set by the Washington State Department of Ecology (370 mg/kg TOC for low molecular weight AHs, and 960 mg/kg TOC for high molecular weight AHs; WAC 173-204-100), it is not overly conservative. Based on threshold values calculated for non-neoplastic liver lesions and DNA damage in English sole, a lower value could be justified. Moreover, the 1000 ng/g dry wt guideline does not incorporate a safety factor, as risk analyses often do, to account for uncertainty in this threshold estimate due to factors such as variations in sensitivity of fish species, or PAH analytes measures, or in ratios of low to high molecular weight PAHs in sediments. We recommend the 1000 ppb threshold as a practical value for making management decisions, which would be protective of estuarine fish populations, but not unworkable from the perspective of sediment remediation and management.

Above the threshold effects concentration of 1000 ppb, the proportion of animals affected and the number of adverse effects observed increases. The degree of increase is modeled by the upper arm of the hockey stick regression, which can be used to estimate the likely degree of injury to marine resources at various sediment PAH concentrations (Table 3). At 5000 ppb, for example, levels of hepatic DNA adducts would be approximately 10-fold the levels found in fish from uncontaminated reference sites, about 30% of the population is predicted to have some form of liver disease, and the number of fish failing to spawn would increase from about 12% to over 35%. At PAH

concentrations of 10,000 ppb, DNA adducts levels would have increased 12-13 fold, 50% of the sole would be expected to have liver disease, nearly 30% of the females would show inhibition of gonadal growth, and over 40% would show inhibition of spawning. This type of information, in combination with data on contaminant effects on other indigenous species, could be used to estimate the loss of productivity or ecosystem services due to PAH contamination at impacted sites.

Summary

Based on the analyses above, we determined threshold sediment PAH concentrations for toxicopathic liver lesions in English sole ranging from 54 to 2800 ng/g dry wt, and a threshold for DNA adducts in liver of 300 ng/g dry wt. Although we do not have sufficient data to statistically determine precise thresholds for other types of injury to English sole, available data indicate that several other types of impairment, including inhibited gonadal growth, inhibited spawning, reduced egg viability, and reduced growth, begin to occur at sediment PAH concentrations in a similar range. Based on these data, a sediment quality guideline of 1000 ppb total PAH (ng/g dry wt) is suggested to protect estuarine fish against several important health effects, including selected degenerative liver lesions, spawning inhibition, and reduced egg viability. With sediment PAH concentrations at or below 1000 ppb, liver lesion prevalences, DNA adduct levels, and growth and reproductive indicators are generally similar to levels observed in English sole from reference sites with minimal sediment PAH contamination, and English sole exhibit little or no toxicopathic injury. Above 1000 ppb, there appears to be a substantial increase in the risk of liver disease and reproductive impairment, as well as potential effects on growth. The proportion of animals affected and the number of adverse effects observed steadily increases, and these relationships can be used to help assess the likely degree of injury to marine resources at various sediment PAH concentrations.

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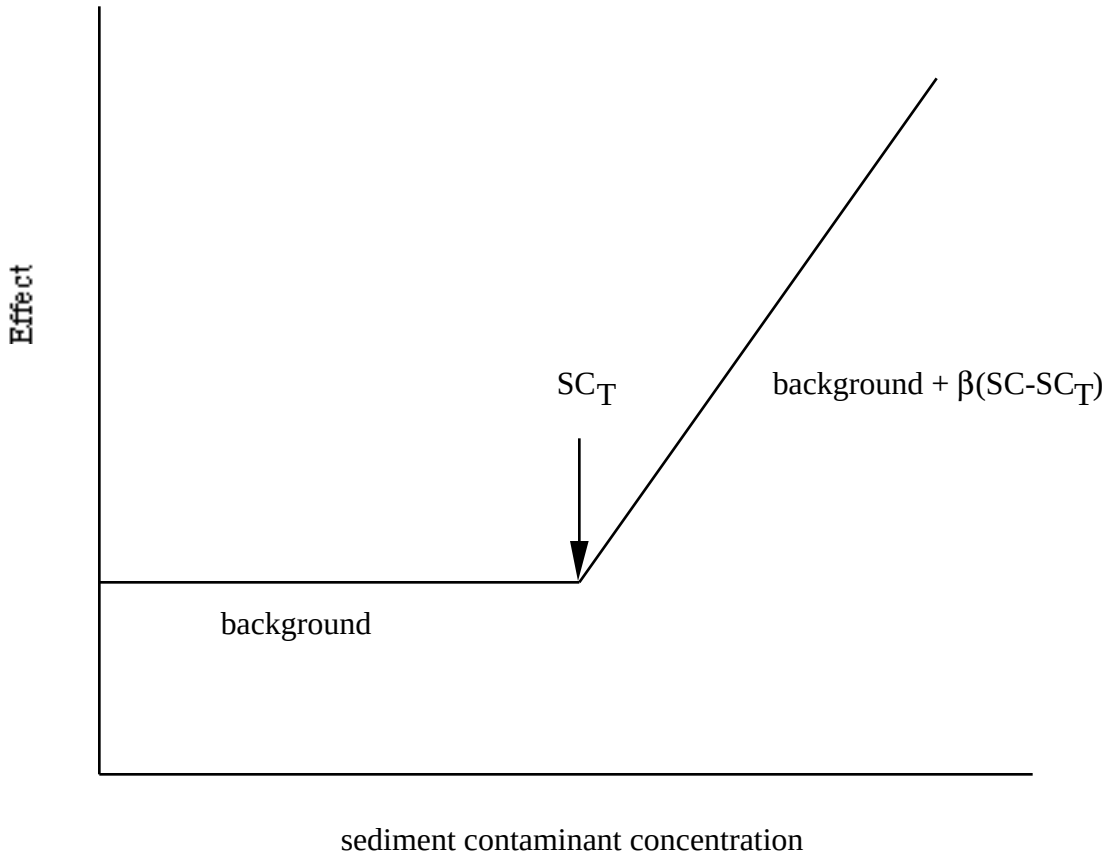

Figure 1. Generalized hocky-stick regression model relating biological effect to sediment contaminant concentration. SC = sediment contaminant concentration, SC_T = theshold sediment contaminant concentration at which the biological effect begins to increase above background level.

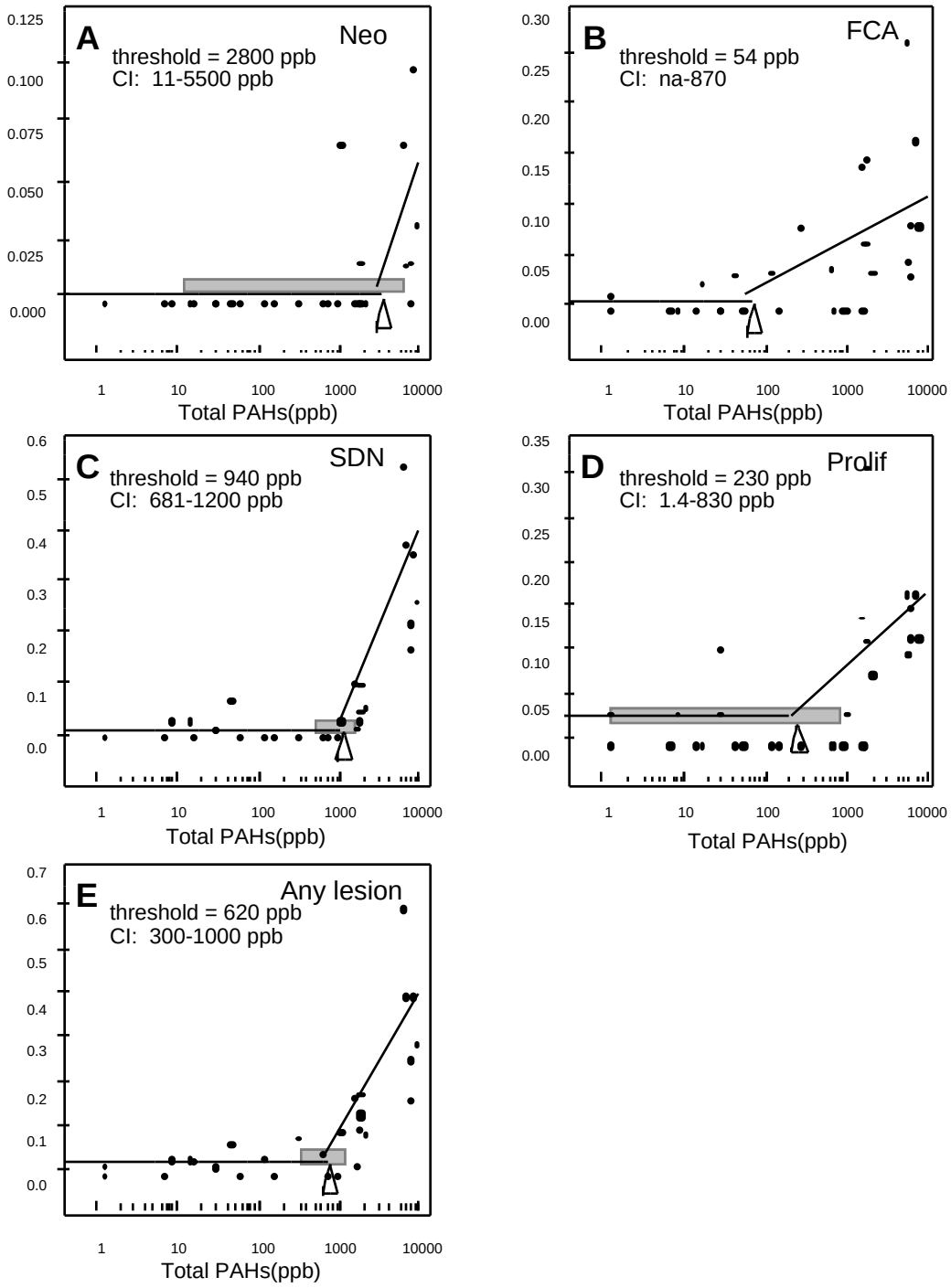

Figure 2. Hockey stick regressions of hepatic lesion prevalence in English sole versus total polycyclic aromatic hydrocarbons (PAH) in bottom sediment in bottom sediment in ng/g dry wt (ppb) for a) neoplasms (Neo); b) foci of cellular alteration (FCA); c) specific degenerative/necrosis (SDN); lesions; d) proliferative lesions (Prolif); e) and either Neo, FCA, or SDN (any lesion). Threshold concentrations are indicated by arrows. Shaded gray bar represents the 90% confidence interval. No lower confidence found was found for the FCA threshold estimate. n = 29. Data from Horness et al. 1998.

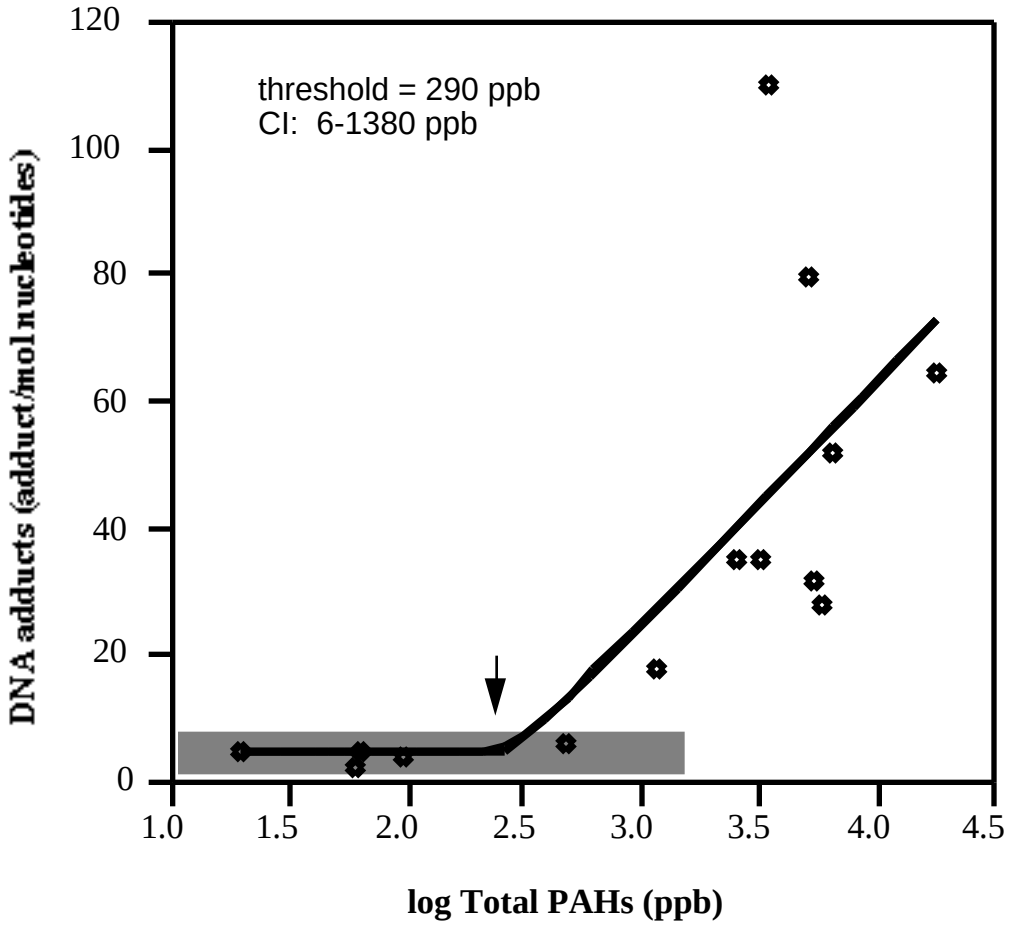

Figure 3. Hockey stick regression of PAH-DNA adducts in liver of English sole versus total polycyclic aromatic hydrocarbons (PAHs) in bottom sediments in ng/g dry wt (ppb) for selected sampling sites in Puget Sound, Washington. Threshold concentration is indicated by arrow. Shaded gray bar represents the 90% confidence interval. Data from Stein et al. 1992; Collier et al. 1998; French et al. 1996; French, unpublished data.

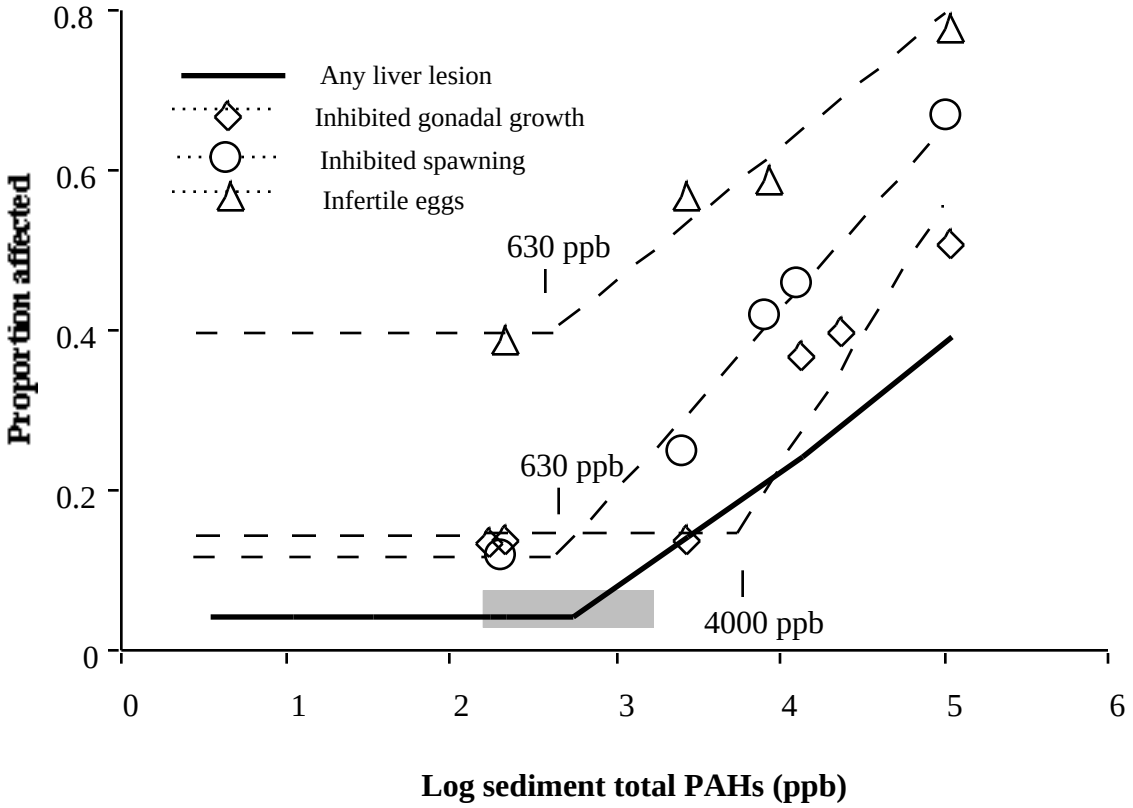

Figure 4. Measures of reproductive function in female English sole plotted against sediment total PAH concentrations at sites in Puget Sound where sole were collected. For inhibited gonadal growth and inhibited spawning, data points represent the proportion of females at the sampling site that exhibited these conditions. For infertile eggs, the data points represent the average proportion of spawned eggs that were infertile in crosses with sperm from reference males for females from the sampling sites. Data are from Johnson et al. 1988; Casillas et al. 1991; Collier et al. 1998; Johnson et al. 1999. Dotted lines indicate the hypothetical hockey stick regression models for reproductive endpoints. The horizontal portion of the curve represents background values for these endpoints, estimated from field data at the reference site with the lowest sediment PAH concentration. Threshold concentrations (indicated by arrows) are the geometric mean of the highest sediment PAH concentration where effect values were at the background level, and the lowest concentration where an increased effect level was observed. The upper portion of the curve is the fitted hockey stick regression model. Estimated threshold and background effect levels were substituted into the model, rather than calculated through regression analysis as for liver lesions. The solid black line represents the fitted hockey stick regression model and effects threshold estimate for Any lesion in English sole (neoplasms, foci of cellular alteration, or specific degenerative necrosis; Horness et al. 1998).

Table 1. Polycyclic aromatic hydrocarbons (PAHs) measured in sediment samples included in determination of PAH effect thresholds for liver lesions in English sole.

Low molecular weight (2-3 rings)

Biphenyl
Naphthalene
1-Methylnaphthalene
2-Methylnaphthalene
2,6-dimethylnaphthalene
Acenaphthene
Fluorene
Phenanthrene
1-Methylphenanthrene
Anthracene

High molecular weight (4-6 rings)

Fluoranthene
Pyrene
Benz[*a*]anthracene
Chrysene
Benzo[*a*]pyrene
Benzo[*e*]pyrene
Perylene
Dibenz[a,h]anthracene

Table 2. Parameter estimates for hockey stick regression of the relationship of sediment polycyclic aromatic hydrocarbon (PAH) concentration (dry weight) with hepatic lesions and reproductive abnormalities in English sole. Adapted from Horness et al. 1998.

	Hockey stick regression parameter estimates				Back-transformed threshold estimates	
Effect[a]	Threshold[b] (log ppb)[c]	Threshold confidence limits (log ppb)[c]	Background[d]	Rate of increase (β)[e]	Threshold (ppb)[c]	Threshold confidence limits (ppb)[c]
DNA damage	2.46	0.75-3.09	4.5	38	288	6-1318
Liver lesions						
Neo	3.45	1.04-3.75	0.004	0.10	2800	11-5500
FCA	1.74 (ns)[f]	na[g]-2.94 (ns)[f]	0.008	0.04	54 (ns)[f]	na[g]-870
SDN	2.97	2.75-3.16	0.013	0.37	940	600-1400
Prolif	2.37	0.14-2.92	0.024	0.09	230	1.4-830
Any lesion	2.79	2.48-3.01	0.024	0.31	620	300-1000
Reproductive abnormalities						
Inhibited gonadal growth	3.6	nd[h]	0.15	0.31	4000	nd[h]
Inhibited spawning	2.8	nd[h]	0.12	0.26	630	nd[h]
Infertile eggs	2.8	nd[h]	0.38	0.19	630	nd[h]
Abnormal larvae	2.8 (ns)[f]	nd[h]	0.25	0.12 (ns)[f]	630 (ns)[f]	nd[h]

[a]For all liver lesions, inhibited gonadal growth, and inhibited spawning, effect endpoints are sampling site prevalences. For DNA damage, endpoint is mean concentration of PAC-DNA adducts in liver of fish from the sampling site. For infertile eggs and abnormal larvae,

endpoints are the average proportion of spawned eggs that were infertile or proportion of larvae that were abnormal in females from Puget Sound sampling crossed with reference males. Neo = neoplasms, FCA = foci of cellular alteration, SDN = specific degeneration/necrosis, Prolif = proliferative lesions), Any lesion = Neo or FCA or SDN, Immature = failing to undergo gonadal development, Non-spawning = failing to spawn; Infertile eggs = proportion of spawned eggs that not fertilized, Abnormal larvae = proportion of hatched larvae with developmental abnormalities

[b]For liver lesions, thresholds are estimated from hockey-stick regression; for reproductive abnormalities, threshold = the geometric mean of the highest sediment PAH concentrations where the effect level was at background and the lowest sediment PAH concentration where an increase in effect level was seen

[c]Measurement units in ppb = sediment PAH concentration in ng/g dry wt sediment

[d]For all liver lesion categories, Immature and Non-spawning, prevalence in frequency of occurence; for Infertile eggs and Abnormal larvae, proportion of eggs or larvae affected. For liver lesions, background was estimated using hockey stick regression; for reproductive abnormalities, background = the effect prevalence or proportion at the reference site with the lowest sediment PAH concentration.

[e]Rate of increase computed as the increase per unit increase in log (PAH concentration in ppb). For reproductive abnormalities, threshold and background effect level estimates from field data were substituted into the hockey stick regression model for computation of β.

[f]ns = not significant; the confidence interval does not lie entirely within the data range, or for β, does not exclude 0. The threshold estimate for Abnormal larvae was not calculated through regression, but is considered non-significant because of the lack of a significant positive correlation between sediment PAH concentration and proportion of abnormal larvae

[g]na = not available; the confidence interval is unbounded in this direction

[h]nd = not determined; sufficient data were not available for calculation of this parameter

Table 3. Estimated effect levels associated with increasing sediment PAH concentration for selected liver lesions and indicators of reproductive function in English sole. For all liver lesions, inhibited gonadal development, and inhibited spawning, the effect level is the proportion of fish estimated to be affected at the indicated sediment PAH concentration; for infertile eggs, the effect level is the proportion of eggs produced by an individual female that are estimated to be unfertile. Effect levels for liver lesions were calculated with hockey stick regression. For reproductive indicators, effect levels at the sampling sites where PAH concentrations were lowest were used to estimate background effect levels (i.e., effect levels at PAH concentrations below 5000 ppb for inhibited gonadal development, and below 1000 ppb for inhibited spawning and infertile eggs).

Liver Lesions

PAH ppb dry wt	neoplasm prevalence	FCA prevalence	SDN prevalence	proliferative lesion prevalence	any lesion prevalence
50	0.00	0.01	0.00	0.02	0.00
100	0.00	0.02	0.00	0.02	0.00
1000	**0.00**	**0.06**	**0.01**	**0.08**	**0.09**
2000	0.00	0.07	0.12	0.11	0.18
3000	0.01	0.08	0.20	0.13	0.24
5000	0.03	0.09	0.27	0.14	0.31
10000	0.06	0.10	0.38	0.17	0.40
100000	0.16	0.14	0.75	0.26	0.71

Reproductive Indicators DNA Damage

PAH ppb dry wt	inhibited gonadal development prevalence	inhibited spawning prevalence	infertile eggs proportion of eggs spawned	adducts per mol nucleotides
50	0.15	0.12	0.38	5
100	0.15	0.12	0.38	5
1000	**0.15**	**0.17**	**0.42**	**25**
2000	0.15	0.25	0.48	36
3000	0.15	0.30	0.51	43
5000	0.18	0.35	0.55	51
10000	0.27	0.43	0.61	63
100000	0.58	0.69	0.80	100