Appendix A: The memorandum prepared by ENTRIX and presented in this appendix is a working review draft which was not edited or finalized by the Trustees.

Appendix A-4:

• A-4, "Risk and Injury Assessment to Fish in Castro Cove"

Original Author: ENTRIX

Distributed to the injury subcommittee in the cooperative NRDA process.



MEMORANDUM

WORKING REVIEW DRAFT

ENTRIX, Inc. 148 Rogers St. Olympia, WA 98512 (360) 352-3225

Date: May 22, 2006

Re: Risk and Injury Assessment to Fish in Castro Cove

Project No. 3054545

SUMMARY

This memo provides an assessment of the potential risks and injury to fish from exposure to mercury and polycyclic aromatic hydrocarbons (PAHs) in Castro Cove sediments. Flatfish (English sole) were assumed as the surrogate for all fish species' risk, in keeping with the analyses conducted for the Hylebos Settlement Agreement (the Hylebos Settlement). Since the Hylebos Settlement did not clarify fish injuries due to mercury exposure, the analysis in this memo estimated mercury uptake (dose) from assumed trophic transfer factors (TTFs), and compared the estimated uptake against tissue-specific screening values in the literature. Principal findings of this analysis can be summarized as follows:

- Flatfish risks from PAH exposure, presuming conditions of the Hylebos Settlement, equated to 20 to 40% service loss, depending on the presumed area where exposure might occur, and assuming the 95% upper confidence limit of the mean sediment concentrations as the exposure point concentration for risk assessment.
- Hazard quotient (HQ) estimations for estimated mercury uptake based on a No Observable Adverse Effect Level (NOAEL) in whole body tissues ranged from 0.53 to 133.5 for fish assumed to occupy the mudflat, 0.31 to 78.5 for fish exposed in the salt marsh, and 0.25 to 63 in the creek channel. (HQ values above '1' are considered at the screening level to be indicative of potential risk and injury).

- Variation in the hazard quotient estimations was the result of profound differences in tissue-based NOAELs reported in the literature.
- High HQ values were all associated with a NOAEL of 0.02 mg-Hg/kg body wt in larval salmonids, from a study that is not widely supported in the scientific community. Values below 1 were associated with an adult fish TRV of 5 mg-Hg/kg-body wt. Intermediate HQ values were associated with TRV values of 0.2 and 0.32 mg-Hg/kg-body wt from literature that is likely the most pertinent to Castro Cove.
- The broad range in the hazards outlined in this memo reflects elements of uncertainty in the modeling of fish risks from mercury exposure in Castro Cove due to the lack of tissue residue data from the site and direct evidence of injury. The high degree of uncertainty in the tissue estimations, and the limited toxicological basis for the use of TTFs for estimating metals doses in general, supports basing the fish injury assessment in Castro Cove on PAH contamination.

BACKGROUND

Estimates of potential impacts to the benthic community have been previously developed through Habitat Equivalency Analysis (HEA) conducted by ENTRIX, Inc., using logistic growth modeling and other models to examine potential mortality based on amphipod bioassay data conducted in association with Tier II sediment investigations (Butcher 3/9/06). Estimates of risks to wildlife from mercury consumption in the Castro Cove vicinity were also provided in an earlier ENTRIX, Inc. memo (Fisher 3/20/06). This memo provides a summary of possible risk to fish, based on fish use of the habitats, and toxicity of the principal contaminants of concern (PAHs and mercury).

Fish Use in Castro Cove

Aquatic habitats available for use to fish in the Castro Cove project area include the waters overlying the cove's mudflats, the lowermost portion of the Castro Cove creek channel, and tidal sloughs within the adjacent Salt Marsh. In past studies of the project area, 21 fish species have been captured (Woodward Clyde 1976, CH2MHill 1982). In the CH2MHill 1982 study, 19 species were captured and identified in the cove's habitats, but species richness and abundance was higher in reference mudflat habitats (Gallinas and Corte Madera) than in Castro Cove during spring sampling. Abundance during the rest of the year was similar amongst the three sites, and diversity in Castro Cove increased to match that of the Gallinas reference site, although it remained lower than the Corte Madera site. Table 1 summarizes the species caught over all seasons in this study. No difference in richness or abundance was observed between the Castro Cove salt marsh habitat and the salt marsh habitats sampled at the reference sites, regardless of season.

(Source CH2MHill 1982)

Fish Species Common Name	Scientific Name	Habitat Where Captured	
Leopard shark	Triakus semifasciata	Creek station only	
Walleye surfperch	Hyperprosopon argenteum	Main channel in cove	
Pacific herring	Clupea harengus	Main channel in cove	
Northern anchovy	Engraulius mordax	All habitats sampled (creek, channel, mudflat, marsh)	
Smelt	Osmeridae sp.		
Whitebait smelt	Allosmerus elongates	Mudflats only	
Topsmelt	Atherinops affinis	All habitats except main channel	
Threespine stickleback	Gasterosteus aculeatus	All habitats except main channel	
Bay pipefish	Syngnatuhus leptorhynchus	Creek station only	
Staghorn sculpin	Leptocottus armatus	All habitats sampled	
Striped bass	Morone saxatilis	All habitats sampled	
Yellowfin goby	Acanthogobius flavimanus	Main channel and salt marsh	
Arrow goby	Clevelandia ios	All habitats except salt marsh	
Longjaw mudsucker	Gillichthys mirabilis	Salt marsh only	
Bay goby	Lepidogobius lepidus	All habitats except salt marsh	
Pacific sanddab	Citharichthys sordidus	Mudflat and salt marsh	
English sole	Parophyrs vetulus	Creek station only	
Starry flounder	Platichthys stellatus	Main channel and mudflat	
White Croaker	Genyonemus lineatus	Mudflat only	

* Capture methods included otter trawl in all habitats except salt marsh; gill net and minnow traps used in salt marsh only.

All of the species listed in Table 1 have potential for exposure to sediment contamination in Castro Cove. The lack of substantial differences in abundance or diversity among the Castro Cove epibenthic and pelagic (midwater) fish assemblages relative to the two reference sites sampled in 1982 suggests that these species are not affected by that exposure. However, the reports did not provide statistical analyses that would definitively clarify significant differences among fish use in the study sites. The most striking finding from the past study was the relative absence in Castro Cove of benthos-associated flatfish, despite the availability of otherwise suitable mudflat habitat. Abundant populations of juvenile English sole and starry flounder were found each spring in the two reference stations, but similar concentrations were not observed in Castro Cove until later, and never at as high an abundance. The authors suggested this finding may reflect possible impacts to these benthosassociated species. However, the lower use of Castro Cove by flatfish and other species in the early spring may also be a result of high spring run-off related to basin hydrology. The report did not include hydrological or water quality comparisons to help clarify the reason for the difference in abundance.

Two other studies have examined fish use of Castro Cove that provide data of additional relevance to the Castro Cove injury assessment. A Woodward-Clyde study (1976) examined fish populations in Castro Cove, the Castro Cove creek channel, and mudflats using trap lines and gillnets. In that study, only four species of fish were captured (starry flounder, American shad, staghorn sculpin and black perch) and numbers were low (N = 23). Sampling methods and effort were not adequate to fully determine fish use in the cove. In a later study of the outer Castro Cove area, outside the NRDA project area, sampling was much more extensive (Entrix 1989). In this latter study, seven stations were sampled at monthly or bimonthly intervals using an otter trawl, over a year long period. Seven species dominated the 12,785 fish captured, with nearly 45 percent derived from two shallow water transects, and 49% from intermediate depth stations. In contrast to the CH2MHill study, roughly 40% of the total catch was flatfish (English sole and speckled sanddab), and 47.8% of the total catch was composed of a mix of shiner surfperch, yellowfin goby, staghorn sculpin, plainfin midshipman and northern anchovy. Similar to the CH2MHill study, abundant English sole were not abundant until the beginning of March, when abundant young of the year captures increased dramatically in the shallow water transects. Taken collectively, these two studies captured four additional species that were not seen in the CH2MHill studies (speckled sanddab, black perch, American shad and plainfin midshipman).

In addition to the fishes identified in the above studies, it is recognized that Wildcat Creek flows into San Pablo Bay north of Castro Cove and supports a limited steelhead trout population; thus, this species should also be considered as a potential user of the project area habitat and is therefore listed in Table 2, although it was not captured in the previous studies.

Fish Toxicity Reference Values

Mercury

Although mercury bioaccumulation in fish has been extensively examined in San Francisco Bay and elsewhere to support human health screening (Greenfield et al. 2003), relatively little has been reported on the effects of mercury on fish themselves, and most that has been reported is from freshwater environments (Weiner and Spry 1996). Effects data on fish populations within San Francisco Bay burdened with mercury are particularly lacking (Davis et al. 2003). Developing a mercury TRV for the protection of fish in Castro Cove is further complicated by the general lack of sediment-associated effects in estuarine fish studies specific to mercury. As reflected in the brief summary of the effects literature appended to this memo, wet weight residues of 6 to 20 µg/g-muscle will likely lead to adverse effects in adult fish. Weiner and Spry (1996) have suggested a no observable adverse effect level (NOAEL) concentration of 5 µg/g-muscle or brain for salmonids based on the earlier work of McKim et al. (1976), where brook trout were chronically exposed for three generations to waterborne methylmercuric chloride. Birge et al (1979) proposed a NOAEL for early life stages of salmonids more than two orders of magnitude below the adult TRV--0.02 $\mu g/g$. based on results from exposing eyed rainbow trout eggs and larvae to mercuric chloride in sediment and water; this TRV is the lowest identified in the literature and is not largely accepted. Snarski and Olson (1982) proposed a NOAEL TRV for fathead minnow reproduction of 0.32 µg/g. This TRV may be more relevant to the aquatic habitats of Castro Cove, given the chronic exposure of the waterborne exposure test (41 weeks), and the estuarine fish species tested.

In a recent study, four analytical approaches of increasing complexity (simple ranking, empirical percentile, tissue threshold-effect level [t-TEL], and cumulative distribution) were evaluated for deriving protective levels of mercury in fish (Beckvar et al. 2005). In this evaluation, a total of 10 papers containing mercury residue-effect information for eight fish species were identified from which paired no-effect residue (NER) and low-effect residue (LER) values were obtained (i.e., equivalent to NOAEL and LOAEL TRVs). The same datasets were analyzed using all four approaches or methods. The reasonableness of the estimated threshold-effect concentrations for the four methods was assessed by comparing them to both the geometric means of control organisms reported in the papers and to ambient tissue residue concentrations from fish captured in areas unaffected by point sources of contaminants. Of the four approaches evaluated in this study, the t-TEL approach--the same approach as outlined in this memo--best represented the underlying data and resulted in a mercury t-TEL of 0.21 mg/kg for adult fish. A mercury t-TEL was not developed for early life stages (ELS) due to the paucity of data. Indeed, the authors indicated that additional ELS fish studies using lower mercury detection limits are needed to validate the protective concentration of 0.02 mg/kg proposed by Birge et al. (1979) discussed in the preceding paragraph.

PAH

Unlike the situation with mercury studies, risk to flatfish from exposure to PAHs has been identified in an array of studies conducted during the previous two decades, which demonstrated significant cellular, reproductive, or other health-related effects in a dose-dependent manner (Myers et al. 1994, NOAA 1997, Johnson 2000). The following conclusions were drawn relative to these past studies on PAH contamination:

- Nearly 10% of English sole examined had cancerous and precancerous lesions in soft body tissue when PAH concentrations were about 1 mg-HPAH [high molecular weight PAH]/kg-sed (dry wt).
- Nearly 5% of adult female flatfish were infertile at about 1 ppm.
- Lesions increased roughly three-fold when sediment HPAH concentrations increased to about 5 ppm (17% above baseline reference areas).
- Invertebrate populations, as measured through the array of Apparent Effects Threshold (AET)¹ bioassays that the State of Washington has used to establish its Sediment Management Standards (SMS), begin to show impacts at about 7.9 ppm.
- At total sediment HPAH concentrations of 10 ppm, over 40% of English sole studies exhibited lesions, and 25% were infertile.
- Between 10 and 69 ppm, more than half of the invertebrate bioassays revealed adverse effects.

¹ AET tests include: (1) bivalve AET, (2) benthic community AET, (3) Microtox AET, (4) amphipod AET, (5) echinoderm AET, (6) oyster AET, (7) Neanthes AET

• A total sediment HPAH concentration of 100 ppm, over 70% of all English sole studied in Puget Sound exhibit toxicopathic lesions, half of adult females have inhibited gonada growth, 2/3rds do not spawn, and at least 3/4ths are infertile; all invertebrate AETs are exceeded.

METHODS

PAH

Consistent with the Hylebos Waterway Natural Resource Damage Assessment in Puget Sound and recommendations from the Trustees, estimates of potential risk to fish from exposure to PAHs were based on English sole. The English sole is representative of a typical flatfish guild species that would use mudflat habitat, for which contaminant uptake could be expected to be significant given their demersal life style, and for which significant toxicological literature on PAHs is available upon which to base injury assessments (Collier et al. 1997, Johnson 2000, NOAA 2002).

It was assumed that potential routes of exposure to PAH contaminants in the mudflat include:

- ingestion of contaminated prey
- incidental ingestion of contaminated sediment
- transdermal exposure from direct contact with contaminated sediments
- bioconcentration across the gills and skin from PAHs dissolved in water

Estimates of potential impact on fish species from exposure to PAH concentrations in sediment were calculated using methods originally outlined in Appendix D of the Hylebos Natural Resource Damage Settlement Proposal (Wolotira 2002). In that proposal, PAH compounds were separated into groupings of low and high molecular weight, but estimates of potential impacts in the Hylebos study were based on HPAH concentrations because total PAHs were not provided in the AET data set from which effects data were derived.

Tier II sediment source data from the Tier II Castro Cove study used the same PAH groupings, with the exception that fluroanthene was listed as a low molecular weight PAH. To maintain consistency with the Hylebos methodology, all fluroanthene results from Castro Cove were switched to the HPAH grouping. Concentrations of each HPAH and LPAH (low molecular weight PAH) were added for each sample to determine the total HPAH and LPAH numbers, respectively. Only the total HPAH number was used to calculate estimates of potential impact for reasons previously mentioned.

The service loss estimates for total HPAHs identified in the Hylebos Settlement and adopted for this draft memo were as follows:

- 20% service loss (flatfish injuries and invertebrate AET) between sediment concentrations from 1 to 8 ppm total HPAH
- 40% service loss from 8 to 17 ppm HPAH
- 60% service loss from 17 to 70 ppm

• 80% service loss when HPAH concentrations exceed 70 ppm

Mercury

A different method for estimating risk and injury to fish from sediment mercury was required than was applied to the Hylebos Settlement for PAHs, as Hylebos mercury injuries were based solely on invertebrate injuries identified through the AET database. However, there have been no tissue samples collected from fish within Castro Cove to compare against the tissue-specific risk screening levels discussed earlier. To estimate a tissue concentration in flatfish inhabiting Castro Cove, it was therefore necessary to assume trophic transfer from the sediment to the fish. In a review of over 300 papers, trophic transfer factors (TTFs) in the literature for total mercury varied widely, with marine TTFs ranging from 0.2 to 6.8, depending on the food web modeled (Suedel et al. 1994). The only study identified in that review which examined trophic transfer from sediment associated benthos to fish was that of Kiorboe et al. (1983), in which a TTF of 1.0 was identified from polychaetes to flatfish, eel and/or eelpout. In the absence of site-specific data, and for the purposes of this memo, tissue concentrations were modeled based on an assumed TTF from sediment to benthos of 1.67, the TTF previously applied to the wildlife risk assessment memo for Castro Cove (Fisher 3/20/06).

For the sake of comparison with the PAH analysis, the following injuries to benthos were identified from sediment mercury in the Hyleobs Settlement from AET bioassays:

- 5% service loss at mercury sediment of 0.41 ppm dry wt (Microtox AET)
- 10% service loss at 1.3 ppm sediment mercury (neanthes AET)
- 15% service loss at 1.4 ppm (echinoderm AET)
- 20% service loss at 2.3 ppm (amphipod AET)

RESULTS

Table 2 presents the sediment exposure point concentrations for PAH and mercury used for screening fish risks.

Contaminant of Concern	Mudflat Surface	Salt Marsh Surface	Castro Cove Creek Surface
Mercury mg/kg	0.963	0.564	0.451
Total PAH mg/kg	14.035	1.53	1.158
Total HPAH mg/kg	13.748	1.375	1.052

Table 2. Upper 95% C.I. of Castro Cove Sediment Data (ppm)

РАН

Using the Hylebos screening and injury estimation methods, the total HPAH surficial sediment in each habitat area in Castro Cove would be associated with some degree of potential service loss. In the Hylebos, over 40% of English sole examined exhibited lesions,

and 25% were infertile at total sediment HPAH concentrations of 10 ppm. If the upper 95% confidence interval (C.I.) concentration of total HPAH contamination in the Castro Cove mudflats (i.e., 13.75 ppm) is assumed to represent the sediment concentration to which all flatfish would be exposed in the Cove, then a significant increase in toxicopathic lesions and reduction in fertility in English sole could be possible. Using the Hylebos Settlement injury breakdown, sediments from the Castro Cove mudflat would equate to service losses of 40%. Based on the lower sediment HPAH concentrations (Table 2) a 20% service loss would be anticipated in the salt marsh and creek channel, respectively.

Mercury

An assumed TTF of 1.67 from sediment to benthic invertebrates, and subsequently from invertebrates potentially ingested by flatfish (i.e., primary consumer to secondary consumer), yielded estimated (assumed) whole body tissue mercury concentrations of 2.69, 1.57, and 1.26 ppm in flatfish presumed to be foraging exclusively in the mudflat, salt marsh and creek channel, respectively. Hazard quotients based on a range of TRVs reported in the literature are summarized in Table 3. These screening values, based on modeled fish tissue concentrations that might accumulate in resident flatfish consuming diets exclusively from each of the Castro Cove sediment study areas, and assuming 100% assimilation, do not indicate significant concern for mercury risks to adult fish, but suggest potential risks for fish reproduction/early life stages may be possible and injury may be occuring.

As an aside, it is interesting to note that if benthos injuries from mercury in Castro Cove were consistent with the Hylebos Settlement, service losses would range between 5 and 10 percent for each of the sediment contamination areas.

Species/Life	TRV	Mudflat	Salt Marsh	Creek Channel
Stage/Chronic Effect	(µg-Hg/g-tissue)	HQ	HQ	HQ
Rainbow	NOAEL: 5	0.53	0.31	0.25
trout/Adult/	(McKim et al.			
Mortality	1976)			
Rainbow	NOAEL: 0.02	133.5	78.5	63
trout/Eggs & Larvae/	(Birge et al. 1979)			
Mortality				
Juvenile and Adult fish/growth & reproduction	NOAEL: 0.20	13.35	7.85	6.3
	(Beckvar et al. 2005)			
Fathead	NOAEL 0.32	8.34	4.91	3.94
Minnow/Larvae/	(Snarski and			
Growth &	Olson 1982)			
Reproduction				

Table 3. Hazard Quotient Risk Characterization Based on a Range of Tissue-Specific TRVs in Fish

Uncertainty

Numerous sources of uncertainty in the assessment of mercury exposure in Castro Cove bring to question the validity of any results based on modeling without site specific data. McGeer et al. (2003) have argued that bioaccumulation factors for metals are inherently flawed in general because conclusions can be reached that have no basis in the toxicological data. Specifically, high BAF values are obtained when exposure concentrations are lowest (suggesting high hazard), and BAF values are lowest when exposure media concentrations are highest (suggesting low hazards). Certainly this relationship is seen when BAF is plotted against sediment mercury from the SFEI reference samples previously provided (*see* Fisher 3/20/06).

Sources of uncertainty specifically include:

- The toxicological foundation for the TTF applied to mercury.
- The mercury uptake model outlined above conservatively assumes 100 percent assimilation from the diet, although that degree of assimilation is far above any reported assimilation rate.
- Tissue doses do not assume any significant uptake from waterborne mercury.
- TRV values were based on freshwater fish studies in controlled laboratory settings.
- Fish use data from Castro Cove suggest significant use in the Cove by juvenile flatfish.
- Lack of tissue data from fish resident to the Cove.
- Lack of information on percent of site use by flatfish relative to total life history.

APPENDIX

Overview of Mercury Effects in Fish

Existing lab and field study reports on mercury toxicity in fish indicate toxicologic effects occur in the same tissues as seen in higher vertebrates, with neurological and reproductive systems affected to the greatest degree (Weiner and Spry 1996). Ninety to ninety-nine percent of the mercury measured in fish tissues has been shown to be in the methylated form (i.e., methylmercury), despite the fact that almost all mercury found in sediments and water is present in other forms (Bloom 1992). There are two principal reasons for this difference: (1) the principal route of exposure to mercury in fish is considered dietary (and zooplankton and other fish food sources also bioconcentrate the methylated form), and (2) solubilized methylmercury also has much greater assimilation efficiency across the gills than inorganic mercury. However, the route of uptake has no bearing on the toxicological significance of methylmercury, as the mode of action will be on internal organs (e.g., brain), not on the tissues exposed directly to waterborne forms assimilated by the fish.

Fish captured in field studies from Minimata Bay, Japan, where mercury was discharged with waste sludge from an acetaldehyde plant, presented a range of toxicological and neurological effects, including diminished locomotor activity, toxicopathic brain lesions, and emaciation (Takeuchi, 1968). Toxicologically-affected fish of six species captured from the Minimata Bay contained an average of 15 ug mercury/g-wet weight in axial muscle (range 8.4 to 24 μ g/g -muscle) (Kitamura 1968).

McKim et al. (1976) examined effects of mercury in three sequential generations of brook trout. Lethal aqueous concentrations of methylmercuric chloride caused loss of appetite, muscle spasms, and deformities prior to death, and yielded tissue concentrations of 24, 32, 42, 48, 147, 58 and 155 ug-Hg/g-tissue in axial muscle, gonad, brain, gill, kidney, liver and spleen, respectively.

Three- to eight-year old northern pike from mercury-polluted Clay Lake in Ontario contained from 6 to 16 μ g/g -muscle, were emaciated, and exhibited a complex of bioenergetic indices of stress including low fat stores, total protein, glucose, and serum alkaline phosphatase. When fish from Clay Lake were transferred to a reference lake and measured a year later, these indices had recovered to approximately half the base line of the reference population, but only 30% of their mercury body burden had been eliminated (Lockhart 1972).

Studies conducted on rock bass in a Virginia stream examined physiological condition in a population residing in a relatively contaminated reach, where the muscle and liver concentrations measured were 1.4 and 2.9 μ g/g-tissue, respectively, versus 0.17 μ g/g and 0.10 μ g/g in fish from the reference reach (Bidweel and Heath 1993). At these tissue concentrations, no significant physiological or biochemical differences were noted between the two populations.

Similar to birds, early life stages of fishes are very sensitive to mercury. Past studies reviewed by Wiener and Spry (1996) have examined mercury-induced teratogenesis in mummichog, rainbow trout, brook trout and fathead minnow. Teratogenesis was observed from laboratory exposures to waterborne mercury at concentrations ranging from $0.2 \mu g/L$ to

100 μ g/L. Craniofacial, cardiovascular and skeletal flexure abnormalities have all been observed (Birge et al. 1979; Weis and Weis, 1991). Exposure of the embryo to waterborne mercury is likely limited by the egg chorion membrane, so the principal route for exposure in the wild is thought to be via translocation during oogensis (Weis and Weis 1991), as the exposure history of the parental female has been reflected in egg burdens in both field (Weis and Weis 1984) and lab studies (McKim et al. 1976). Niimi (1983) found that translocation into eggs from contaminated females in the wild yields relatively lower concentrations of mercury than is found in the tissues of the parent, amounting to roughly 0.3 to 2.3 percent of the whole-body burden. Burdens of 0.04 to .010 ug/g-egg, less than 1% of the body burden associated with overt toxicity in adult rainbow trout, have been identified as the LOEL for eyed eggs or larval mortality after 10 days of exposure (Birge et al. 1979).

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