UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE

Northwest Fisheries Science Center
Environmental Conservation Division
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March 26, 1999

MEMORANDUM FOR: NORCAx11 - Rob Wolotira

FROM: F/NWC5 - John E. Stein John E. Stein

SUBJECT: Hylebos Waterway Fish Injury Assessment - Salmon Laboratory Studies Round III Pilot Study

This is the data report for the Hylebos Waterway Fish Injury Assessment - Salmon Laboratory Studies Round III Pilot Study as described in the Sampling and Analysis Plan (SAP) of October 1998 and recommendations for conducting pilot study 2. The goals of the Hylebos Waterway Fish Injury Pilot Study were to (1) document the palatability of the low-fat pellet that we had made for delivering the contaminants to juvenile fish and (2) to ascertain that those contaminants were bioavailable when administered in this fashion. These essential pieces of information will be used for planning and executing both the second pilot study and the proposed comprehensive study.

Experimental methods

Fish were fed contaminated fish pellets for up to 28 days; a period representative of the residence time for outmigrant juvenile salmon in river systems/estuaries such as the Hylebos Waterway. The time period for exposure was variable because of a protozoan parasite (Cryptobia) that caused premature death in the fish.

Following contaminant exposure, the fish were sacrificed for chemical analysis. Once the analyses were completed, bioaccumulation of the contaminants was assessed in whole fish and compared to the known amount fed to fish. Details of the chemical analyses are provided in the Quality Assurance Plan (QAP), which was submitted previously (Jan 1999).

The polychlorinated biphenyls (PCBs) and chlorobutadienes (CBDs) were measured in the pellets and fish tissue, and the data presented here are evidence of the palatability of the pellets and the degree to which the contaminants were bioaccumulated. PAHs are rapidly metabolized in fish; hence measurement in tissue represents only a small fraction of that taken up. The PAHs were measured

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in a select group of fish for comparison to control values to determine if bioaccumulation occurred from the pellets. The PAH parent compounds were measured in the fish pellets, allowing for a comparison to PAHs in tissue. Whole-body PCB determinations were conducted for all samples and a select number of samples (both replicates for two treatments) were analyzed in detail for 17 individual PCB congeners. Fish tissue was analyzed for CBDs from those treatments that had CBDs in the food.

In this study, we examined 9 doses and two controls (Table 1), which are representative of the range of doses planned for the comprehensive study. The contaminant dosages (PCBs, PAHs, and CBDs) were assessed in this pilot study in the same proportions as those proposed for the comprehensive study. At the end of the exposure period, five fish were taken from each tank replicate to form 1 composite sample per tank for chemical analysis. Therefore, each contaminant dose has two chemical determinations for the sampling period.

The food and fish samples were analyzed as described in the QAP of January 1999. Briefly, aliquots of the food samples and the fish composites were solvent extracted, and the analytes were isolated by fractionating the extract using size-exclusion high-performance liquid chromatography. The analytes were quantified using gas chromatography/mass spectrometry with sequenced selected ion monitoring. All samples were analyzed for Aroclor 1254; selected samples were also analyzed for selected aromatic hydrocarbons, chlorinated butadienes, and/or selected individual polychlorinated biphenyl congeners. Quality control data for each batch of food and fish samples were acquired and evaluated according to the QAP, and the results are discussed in the enclosed Case Narrative. In addition, the SRM 1974a and the trout control material were used to validate the analytical method (see case narrative).

Results and Discussion

The quality of the chemical analyses was established by an initial demonstration of proficiency (see appendix) and by each sample batch's quality control data, all of which met the minimum quality control criteria in the QAP, as described in the case narrative (attached). The measured concentrations of the PCBs and PAHs applied to the pellets were generally very close to the nominal concentration (Table 2). The measured concentrations for the chlorobutadienes (CBDs) were consistently low compared to the nominal concentrations. Measured CBD concentrations were 37 – 38% of nominal, and if corrected for recovery, which averaged 67% for these compounds, the measured to nominal ratio would be 55 to 57%. The CBDs are likely more volatile than the PCBs, hence some of the applied material was probably lost when the solvent that was used to apply the compounds was evaporated. Such a loss does not affect our ability to conduct an experiment with CBDs added to feed at the desired dose.

The fish acquired very high concentrations of PCBs and much less of the PAHs and CBDs (Table 3). We know that the PAHs are extensively metabolized,

and the results in Table 3 are consistent with that finding. Very little is known about the disposition of CBDs in fish tissue. The PCBs were accumulated in a highly linear fashion from the fish pellets (Figure 1), producing a slope of 0.86. The CBDs were also accumulated in a dose-dependent fashion (Figure 2); however, the slope was 0.10. A comparison of this lower slope to the one for PCBs indicates either lower assimilation of CBDs from the pellets, higher metabolism, or a combination of both factors. Even though the exposure time was slightly variable between tanks, there appears to be no effect on the linear regression line for bioaccumulation.

The reason for the elevated PCBs in the solvent-control food is unknown. The PAH and CBD concentrations in food do not show the same large differences between the non-solvent and solvent controls. Moreover, because the PCB concentrations measured in fish tissue for the non-solvent and solvent controls were the same, this indicates that the solvent control food was not contaminated when it was being fed to fish. The elevated concentration in the solvent control food may have come during the sampling or analysis phases of this study. Future studies will have sample replicates that will allow detection of this type of anomalous result.

Data acquired and analyzed as described in the SAP is used here to address the following questions:

1. **Was the low-fat pellet palatable to juvenile salmon and does it allow them to thrive?** The experimental fish lived on the low fat pellets for three months in the laboratory. One month of this period was during the exposure phase of the experiment. No significant rejection of the pellets was observed.

2. **Are the contaminants (PCBs, PAHs, and CBDs) that were applied to the fish pellets and fed to fish accumulated in the tissues?** Figures 1 and 2 show a very high correlation between the dose of PCB or CBD in the food and the concentrations measured in whole fish. The PAHs show very little bioaccumulation and the results were somewhat variable over treatments. A few samples contained PAHs in the 1 µg/g range, which may have been due to small amounts of food remaining in the stomachs of the fish sampled.

   Based on the hydrophobicity of the PAHs, the uptake efficiency of the parent PAHs was probably similar to that observed for the PCBs, but extensive metabolism converted most of the parent compounds in the tissues to metabolites that are excreted. We have proposed to analyze bile for PAH metabolites to assess uptake; however, because of limited numbers of fish available in this study, we did not analyze bile.

3. **Are the tissue concentrations found in juvenile salmon fed contaminated pellets as predicted based on how much was fed to the fish?** Based on the mean fish weight of 20 grams and the amount of food received per day (3% body weight), the amount accumulated over the 28 day period was as predicted. For example, a 20 gram fish receiving 0.6 grams of fish pellets per day (3% body wt.) that contained 10.8 µg/g PCBs, would have received 6.5 µg of PCBs per day. Over 30 days, the total amount would be 194.4 µg. This concentration of PCBs in a 20 gram fish (= 4 grams dry wt.) would equal almost 48.8 µg/g dry weight. Reported assimilation efficiencies for PCBs in these types of studies range from 25% to 75%. If we assume an uptake efficiency of 25%, then the fish eating pellets with 10 µg/g PCBs would be expected to have a whole body dry weight concentration of approximately 12 µg/g, which is very close to the proportion measured. We noticed during this study that not all the food given to the fish at this ration was consumed. Considering that the fish may not have eaten all of their allotment of food and the uptake efficiency is not known, the tissue concentrations can not be predicted precisely, but generally fall within the range of expected concentrations.

Conclusions

This study demonstrates that we are able to dose a low-fat pellet at desired concentrations and that the fish will consume these pellets and accumulate contaminants in their tissues in a dose-dependent fashion. The data from this pilot study on bioaccumulation provide the following recommendations for the proposed pilot study 2 and the comprehensive study that will assess the dose-response relationship between tissue residues and biological effects for these contaminants of interest.

Recommendations:

1. Maintain the dosing scheme for PCBs and PAHs.
2. Measure bile for metabolites of PAHs.
3. Adjust dosage of CBDs in the fish pellets to produce the desired tissue residue.
4. Assure that no food is present in the stomachs by dissecting the stomachs at the time of sampling.

cc: F/NWC5 – J. Meador
F/NWC5 - T. Collier
F/NWC5 - P. Krahn

Table 1. Hylebos Pilot Study - Summary of Chemical Analyses

			Nominal Concentration in food (µg/g)			Food				Fish Day 45			
						PCBs				PCBs			
Treat.	Tank	Contaminant	PCBs	PAHs	CBDs	Tot	Indiv	PAHs	CBDs	Tot	Indiv	PAHs	CBDs
1	1	control								X			
1	2	control				X		X	X	X		X	X
2	3	solvent control								X			
2	4	solvent control				X		X	X	X		X	X
3	5	PCBs	1.2							X			
3	6	PCBs	1.2			X				X			
4	7	PCBs	10.8							X			
4	8	PCBs	10.8			X				X			
5	9	PCBs & PAHs	1.2	25						X			
5	10	PCBs & PAHs	1.2	25		X		X		X			
6	11	PCBs & PAHs	3.6	25						X	X	X	
6	12	PCBs & PAHs	3.6	25		X		X		X	X	X	
7	13	PCBs & PAHs	10.8	25						X	X	X	
7	14	PCBs & PAHs	10.8	25		X	X	X		X	X	X	
8	15	PCBs & PAHs	50	25						X			
8	16	PCBs & PAHs	50	25		X		X		X			
9	17	PCBs, PAHs, & CBDs	1.2	25	1					X			X
9	18	PCBs, PAHs, & CBDs	1.2	25	1	X		X	X	X			X
10	19	PCBs, PAHs, & CBDs	1.2	25	4					X			X
10	20	PCBs, PAHs, & CBDs	1.2	25	4	3*		3*	3*	X			X
11	21	PCBs, PAHs, & CBDs	1.2	25	16					3*			3*
11	22	PCBs, PAHs, & CBDs	1.2	25	16	X		X	X	X			X
Day 0	pool	Baseline for fish→	(these are fish ->)			3		3	3				
		Dataset Totals				16	1	14	10	24	4	6	10

Total number of food and fish composite samples to be extracted =

* Replicate analyses of one composite. X=composite of 5 fish

March 25, 1999

Table 2. Summary results of concentrations in fish pellets

	Nominal food conc (ng/g dry wt.)			Measured food conc (ng/g dry wt.)				
Treatment	Tot PCBs	Tot PAHs	Tot CBDs	1254	Tot PAHs	HCBD	PCBD	Tot CBDs
1	control			24	33			
1	control			24	33			
2	solvent			420	70			
2	solvent			420	70			
3	1200			1500	70			
3	1200			1500	70			
4	10800			12000	42			
4	10800			12000	42			
5	1200	25000		1200	24150			
5	1200	25000		1200	24150			
6	3600	25000		4100	24850			
6	3600	25000		4100	24850			
7	10800	25000		13667	24680			
7	10800	25000		13667	24680			
8	50000	25000		64000	25320			
8	50000	25000		64000	25320			
9	1200	25000	1000	1200	23060	140	230	370
9	1200	25000	1000	1200	23060	140	230	370
10	1200	25000	4000	1333	24850	580	923	1503
10	1200	25000	4000	1333	24850	580	923	1503
11	1200	25000	16000	1100	21290	2300	3800	6100
11	1200	25000	16000	1100	21290	2300	3800	6100

Table 3. Summary results of concentrations in whole fish

	Nominal food conc (ng/g dry)			Measured fish conc (ng/g dry wt.)				
Treatment	Tot PCBs	Tot PAHs	Tot CBDs	1254	Tot PAHs	HCBD	PCBD	Tot CBDs
1	control			140	0			
1	control			150	19			
2	solvent			170	0			
2	solvent			140	16			
3	1200			1400	0			
3	1200			1500	0			
4	10800			9000	0			
4	10800			13000	0			
5	1200	25000		1200	0			
5	1200	25000		1300	193			
6	3600	25000		3300	478			
6	3600	25000		3200	80			
7	10800	25000		10000	26			
7	10800	25000		7600	1218			
8	50000	25000		55000	214			
8	50000	25000		55000	55			
9	1200	25000	1000	1500	1041	42	24	66
9	1200	25000	1000	720	159	25	10	35
10	1200	25000	4000	1500	494	120	70	190
10	1200	25000	4000	1400	976	130	84	214
11	1200	25000	16000	1100	568	350	193	543
11	1200	25000	16000	850	737	480	280	760

Figure 1. PCBs in food and whole fish

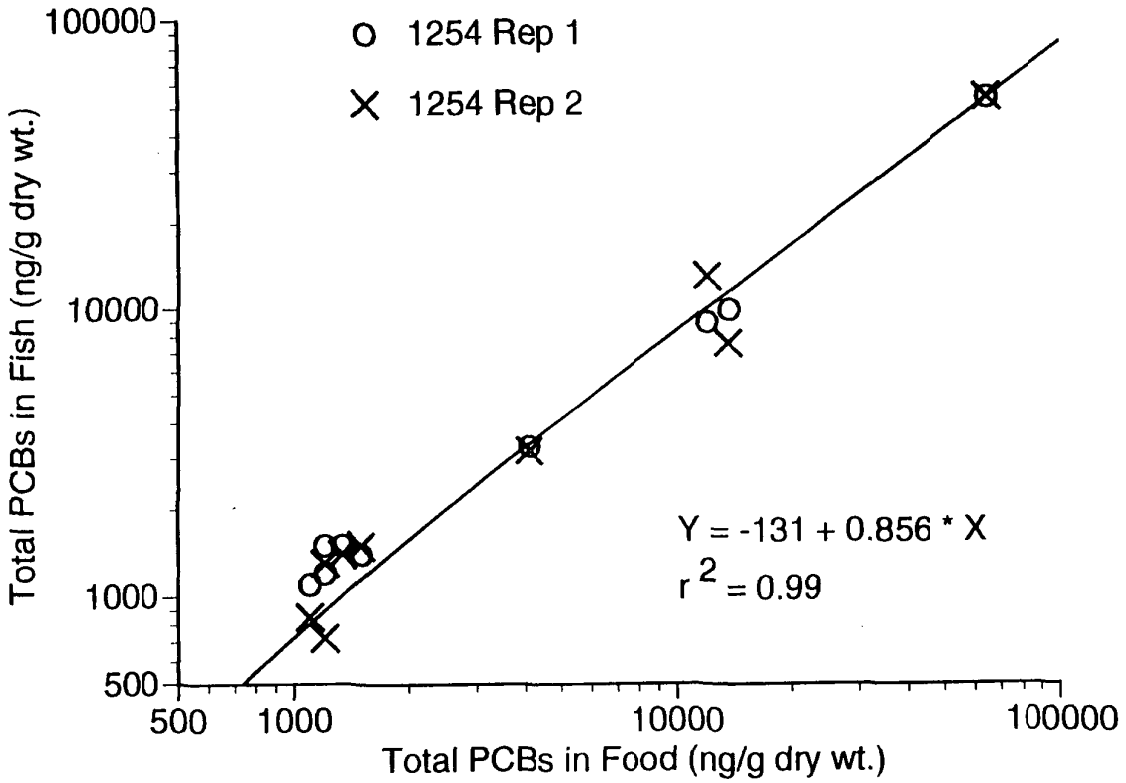


Figure 2. CBDs in food and whole fish

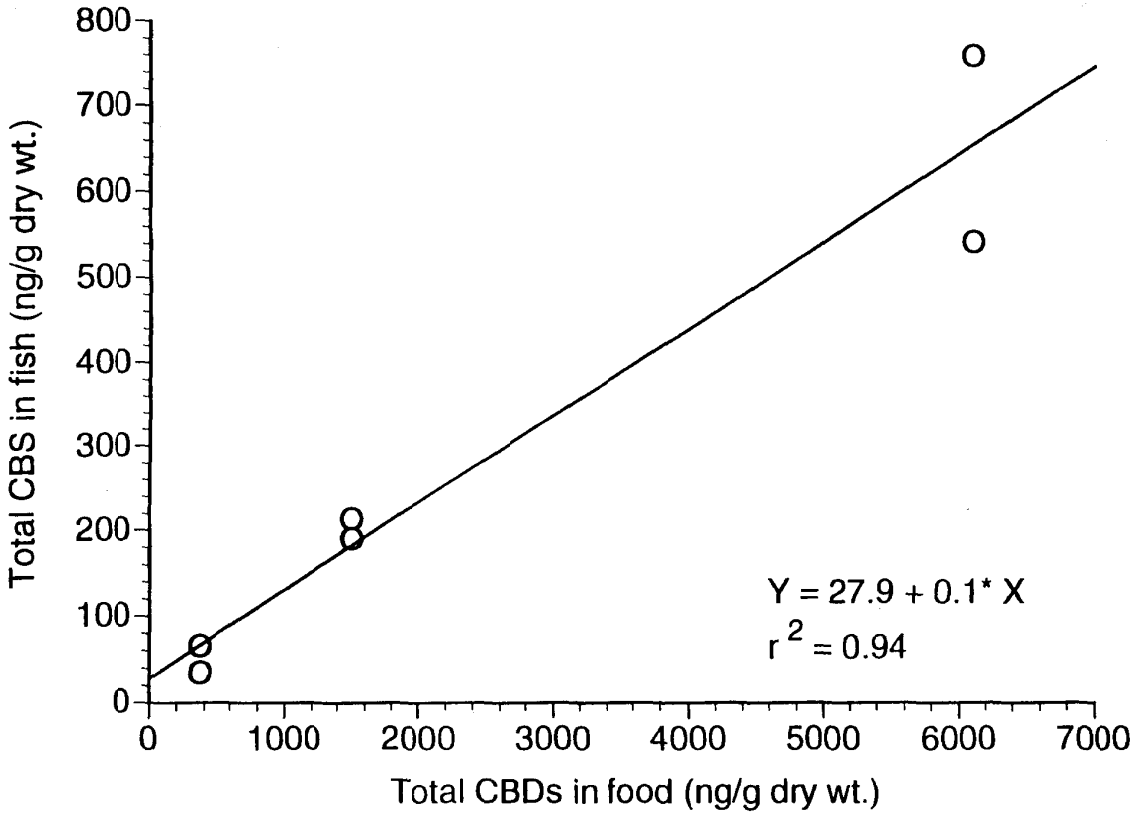
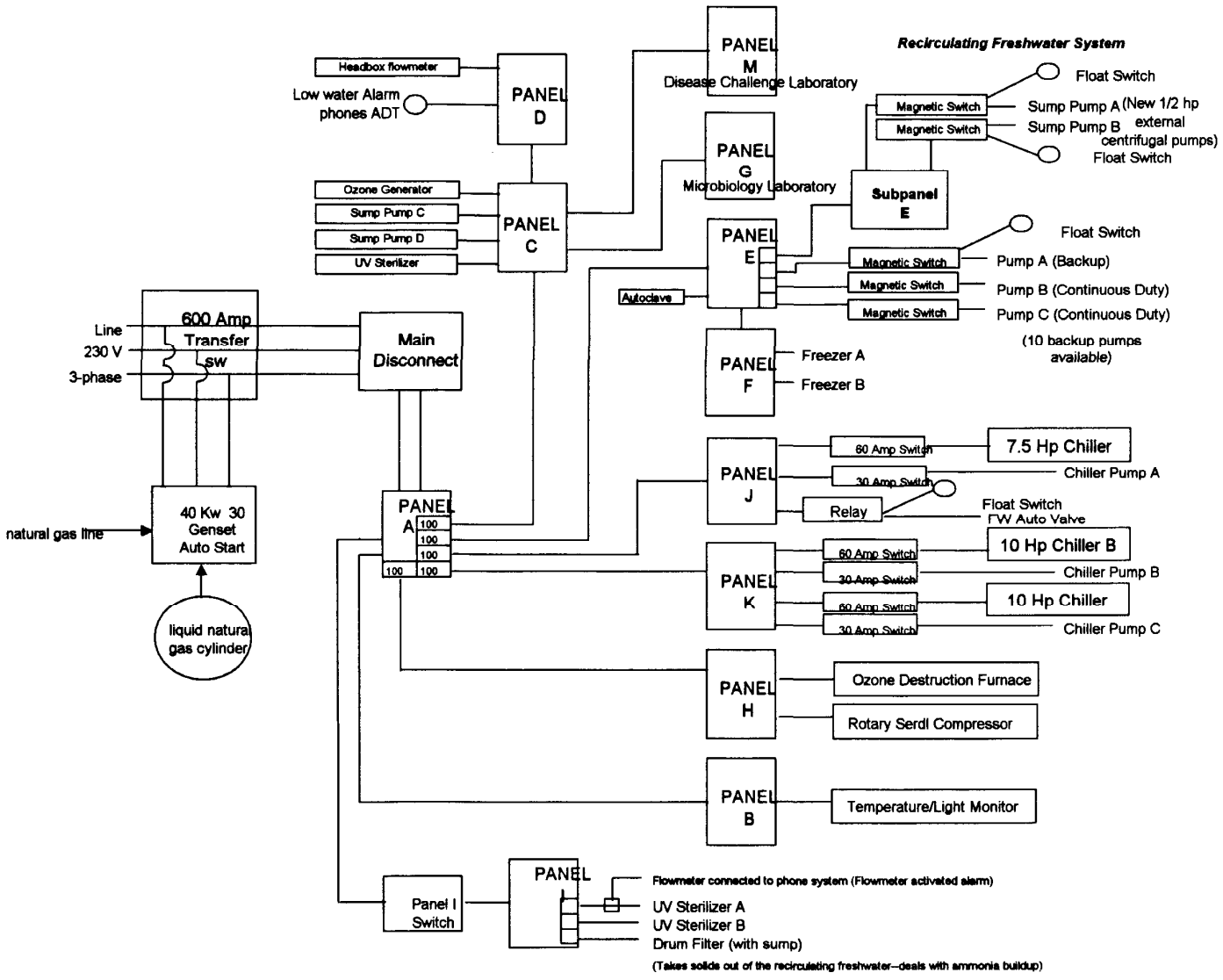


Diagram of the Mukilteo Facility Electrical System Associated with the Round III Pilot Study



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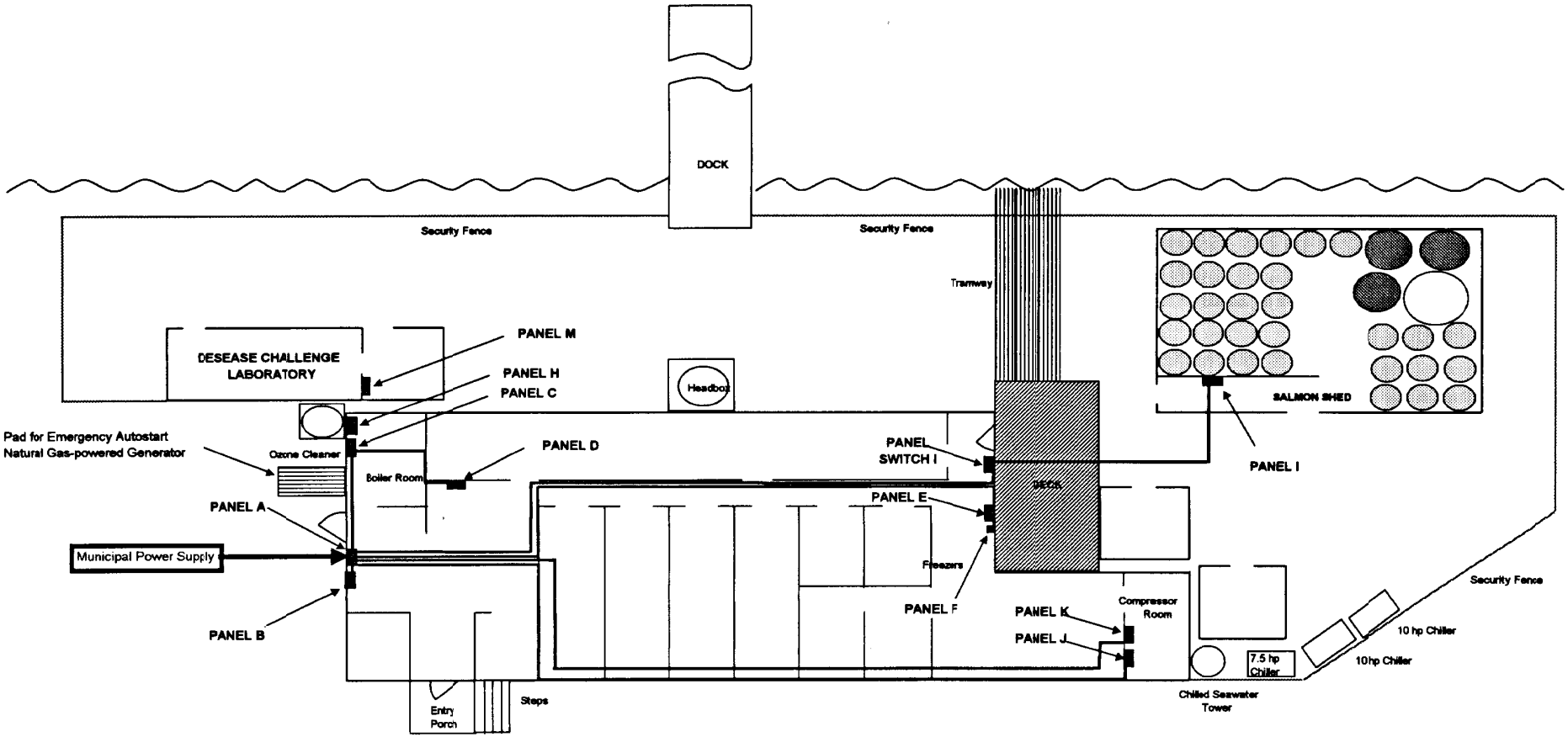
Schematic Diagram of the Electrical System Associated with the Round III Pilot Study



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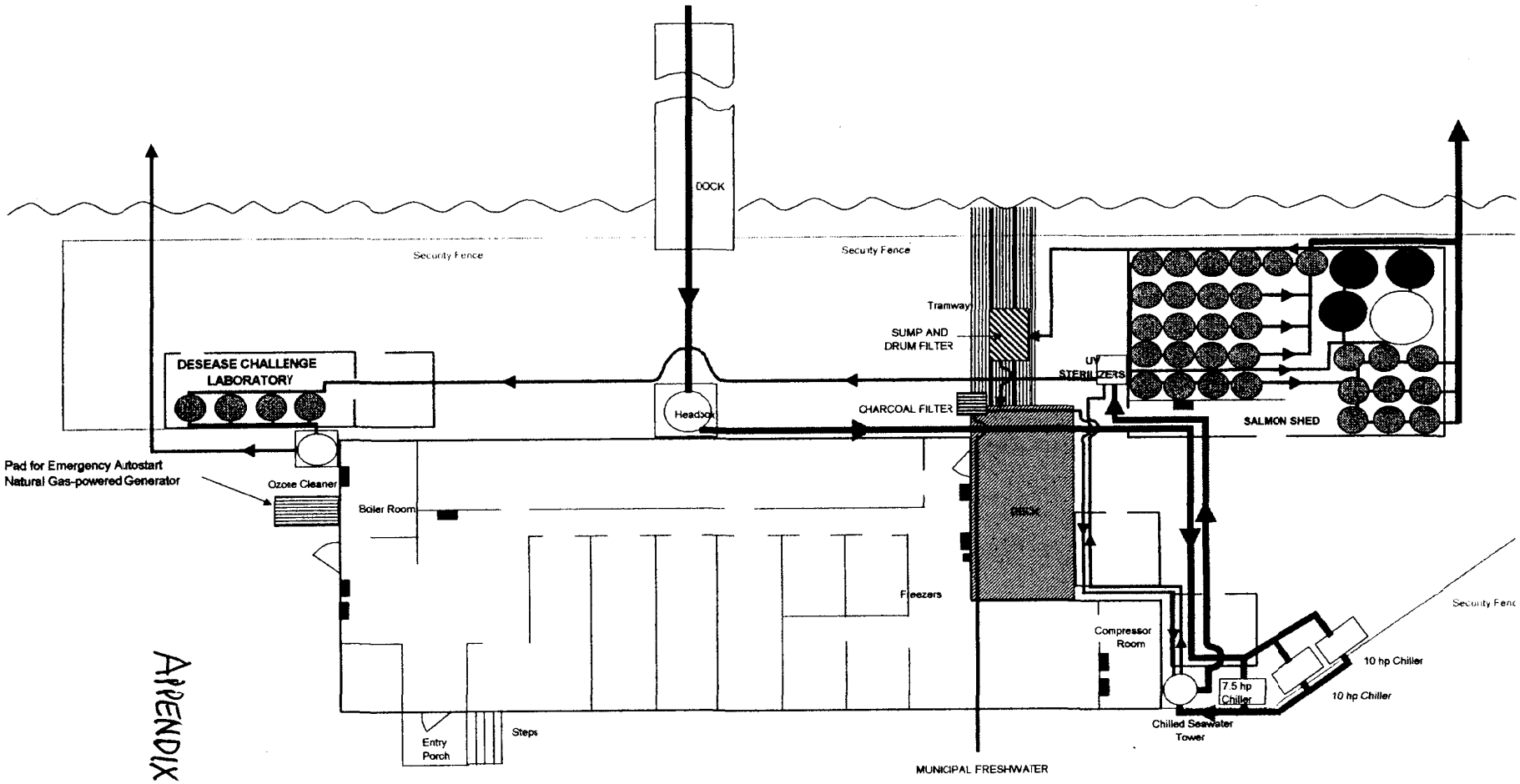
Schematic Diagram of the Circulating Water Systems Associated with Round III Pilot Study



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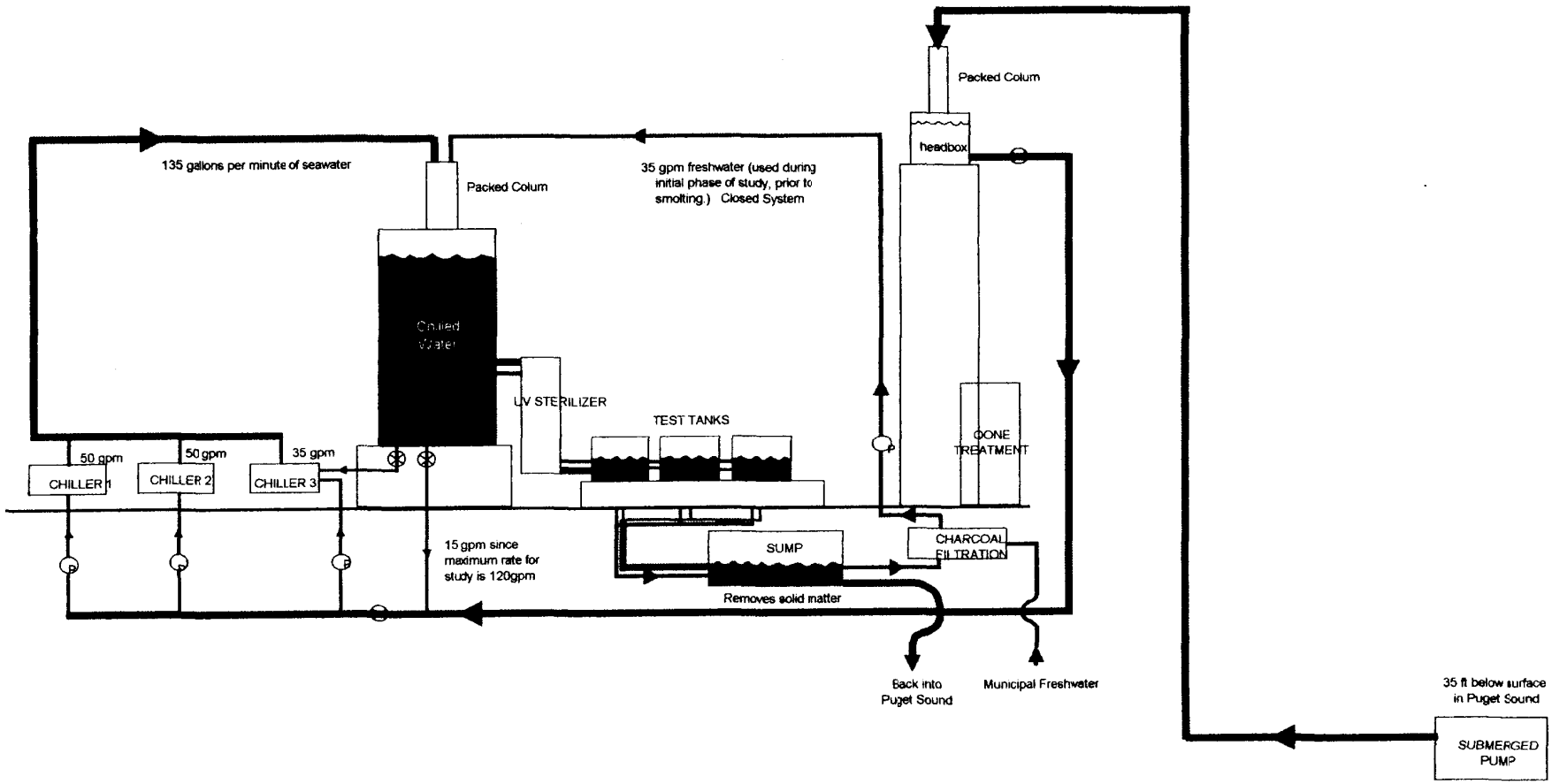

Waterflow Schematic Diagram for the Round III Pilot Study at the NMFS NWFSC Mukilteo Facility