Green - Duwamish Watershed
Endocrine Disrupting Compounds Survey

Sampling and Analysis Plan

Prepared by the
King County Department of Natural Resources and Parks
Green - Duwamish Watershed Water Quality Assessment Program

November 2003

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1 INTRODUCTION

This sampling and analysis plan (SAP) presents project information and sampling and analytical methodologies that will be employed to perform a survey of the nature and extent of endocrine disrupting compounds in the Green - Duwamish (GD) watershed. The SAP includes a description of the project, study design, sampling and analytical methodologies, and project reporting. This work is being performed as part of the King County Wastewater Treatment Division's ongoing efforts to monitor the health of the environment in its major watersheds.

1.1 Project Background

Endocrine disrupting compounds (EDCs) are chemicals that mimic natural hormones, inhibit the action of hormones, or alter normal regulatory functions of the immune, nervous, and endocrine systems (King County, 2002a). Municipal wastewater treatment systems are typically viewed as a potential "line of defense" against the release of EDCs to waterways. Although most chemicals are removed through primary or secondary treatment processes, some portion of these chemicals may be discharged in treated effluent.

A variety of potentially endocrine disrupting chemicals are known or suspected to be present in secondary treated wastewater effluent and storm runoff. These chemicals include natural and synthetic hormones, alkylphenolic compounds, phthalates, pesticides, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and some metals. The purpose of this survey is to characterize concentrations of EDCs in the waters of the GD watershed. Separate but similar surveysare being undertaken in 2003 in the Lake Sammamish/Lake Washington watershed and King County marine waters.

This survey will involve the collection and analysis of GD water samples from the mainstem Green - Duwamish River as well as on major tributaries near the mouths of the four main basins. Samples will be collected during storm and baseflow conditions for a period of one year. Analyses will include both standard, instrumental methods for a wide variety of trace organic compounds and immunoassay testing for two hormonal compounds.

1.2 Project Organization and Schedule

The tasks involved in conducting the GD Watershed Endocrine Disrupting Compounds Survey and the personnel who will assume responsibility for those tasks are listed below.

- Doug Henderson – Green - Duwamish (GD) Water Quality Assessment Program doug.henderson@metrokc.gov 206-263-6317. Project management, study design, data validation and analysis, and preparation of final survey report.
- Curtis Nickerson – Taylor Associates. Cnickerson@taylorasso.net, 206-267-1405. Coordination of field activities including preparation of sampling equipment and collection of samples.
- Diane McElhany – Environmental Laboratory diane.mcelhany@metrokc.gov 206-684-2304. Coordination of trace organic laboratory analyses.
- Dr. Jim Buckley - Environmental Laboratory jim.buckley@metrokc.gov 206-684-2314. Coordination and analyses for ELISA testing.
- Fritz Grothkopp – Environmental Laboratory fritz.grothkopp@metrokc.gov 206-684-2327. Coordination of all Environmental Laboratory activities, project data review, and data reporting

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- Betsy Cooper – Wastewater Treatment Division betsy.cooper@metrokc.gov 206-263-3728. Review of study design, SAP, and final survey report.

The anticipated project schedule is:

January 2003 - First sampling event
December 2003 - Final sampling event
October 2004 - Data review and draft report
December 2004 - Final report

2 Study Area Description

The Green-Duwamish Watershed includes a drainage area of approximately 484 square miles of varied terrain and land use from forested headwater areas at the crest of the Cascade Mountains to industrial and port facilities of the Duwamish estuary. The project study area encompasses the Green-Duwamish watershed from the Tacoma Diversion Dam at river mile 61 to the mouth of the Duwamish River at Elliott Bay (Figure 1), about 261 square miles. The upper Green River Basin (231 square miles) is not included in the study area. The upper portion of the watershed (47% of the basin) is comprised of mountainous, forested areas that drain to Howard Hanson Reservoir where the US ACOE operates a dam to control floods and augment in-stream flows. Downstream of the dam, the City of Tacoma diverts water for municipal supply from reservoir inflows that are passed through the dam. The middle portion of the watershed drains 35% of the total basin area. Major tributaries in the middle portion include Newaukum Creek draining the Enumclaw plateau and Soos Creek draining the Covington upland. Land use/land cover is mixed and includes forests, agricultural, residential, and commercial areas. The lower portion of the watershed (18% of the basin) is dominated by Green-Duwamish valley. Land use/land cover is mixed but includes substantial commercial and industrial areas within the cities of Auburn, Kent, Renton, Tukwila, and Seattle.

Summer flows in the river, gauged at Auburn, are in the range of 250 cubic feet per second (cfs). Winter flows average about 1,500 to 2,000 cfs, with peaks of more than 5,000 cfs during storm events. The study area has a population of about 324,000 (King County 2000). Extensive population growth is occurring in the Soos Creek basin in unincorporated King County and within the cities of Kent, Covington and Maple Valley. Salmonid species present in the watershed include chinook, chum, coho, sockeye, bull trout and cutthroat trout (King County 2000).

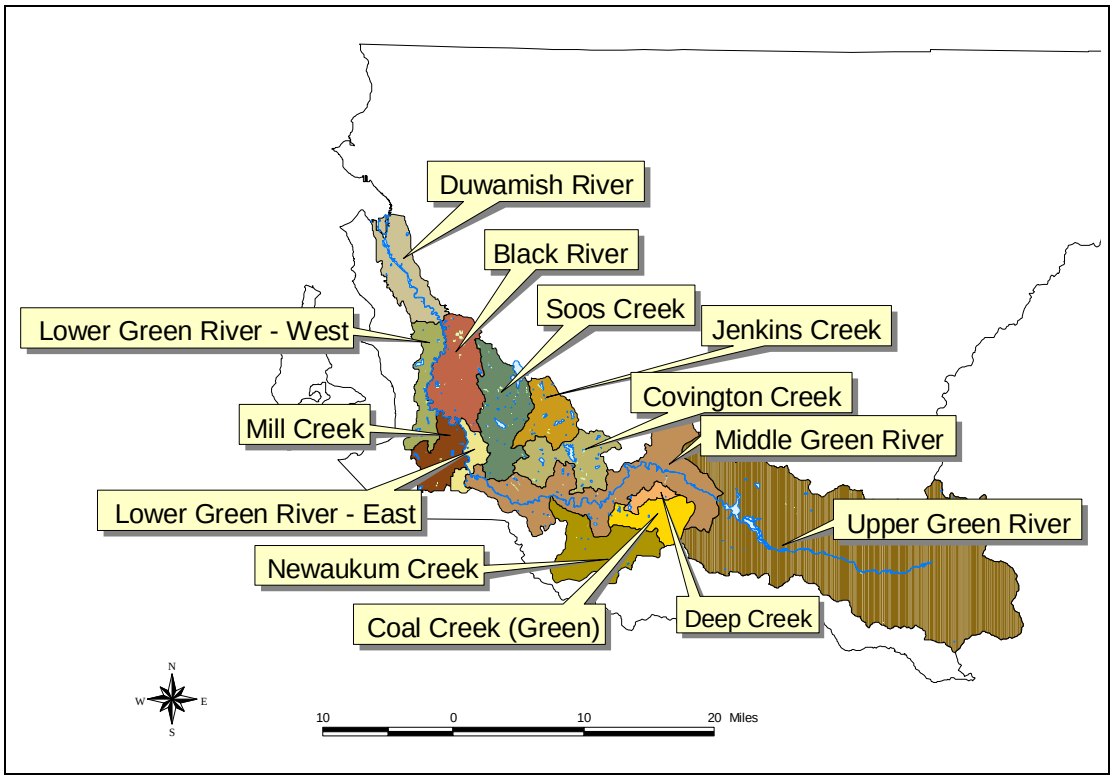

Figure 1. The Green - Duwamish Watershed with the sub-basins labeled

3 Study Design

The primary goals of the GD Watershed Endocrine Disrupting Compound Survey are to:

- characterize the nature and extent of potentially endocrine disrupting compounds in the waters of the Green/ Duwamish watershed; and
- evaluate spatial and temporal variations of those compounds that are detected.

A secondary survey goal is to validate the use of immunoassay testing both as a weight-of-evidence approach for evaluating sample results generated by standard, instrument analysis and to assess the potential of using these relatively low-cost, rapid assays as a reliable tool for measuring concentrations of hormonal compounds in natural waters.

3.1 Data Quality Objectives

The data quality objectives (DQOs) of this survey are to collect data of sufficient quantity and quality to meet the survey goals. Statistical analysis of data collected for this survey will be performed to evaluate whether a sufficient quantity of data has been collected to meet the survey goals.

The survey goals are to characterize water concentrations of various trace organic compounds at different locations and to evaluate any differences between sites, either spatially or temporally. It is anticipated that many organic compounds will not be detected in ambient Green – Duwamish Watershed water. Statistical analysis of data that are generally "undetected" will use binomial calculations on the probability of a sample with a detectable concentration of the organic compound and the probability of finding two and three samples in succession with detectable values at a given site. Statistical analysis of data for those organic compounds that are detected regularly or occasionally will be accomplished through the use of medians and interquartile ranges.

Validation of project data will assess whether the data collected are of sufficient quality to meet the survey goals. The data quality issues of precision, accuracy, bias, representativeness, completeness, and comparability are described in the following sections.

3.1.1 Precision, Accuracy, and Bias

Precision is the agreement of a set of results among themselves and is a measure of the ability to reproduce a result. Accuracy is an estimate of the difference between the true value and the determined mean value. The accuracy of a result is affected by both systematic and random errors. Bias is a measure of the difference, due to a systematic factor, between an analytical result and the true value of an analyte. Precision, accuracy, and bias for analytical chemistry and immunoassay testing may be measured by one or more of the following quality assurance/quality control (QA/QC) procedures:

- collection and analysis of field replicate samples (field replicate results should exhibit a relative percent difference less than 50% in order for the evaluation of the spatial and temporal chemical concentrations to be meaningful); and
- analysis of various laboratory QC samples such as blanks, spikes, and replicates.

3.1.2 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point, or an environmental condition. Water samples will be collected from stations with predetermined coordinates to represent specific site conditions, both compared to other locations and at each location over time.

3.1.3 Completeness

Completeness is defined as the total number of samples analyzed for which acceptable analytical data are generated, compared to the total number of samples submitted for analysis. Sampling at stations with known position coordinates in favorable conditions, along with adherence to standardized sampling and testing protocols will aid in providing a complete set of data for this project. The goal for completeness is 100%. If 100% completeness is not achieved, the project team will evaluate if the data quality objectives can still be met or if additional samples may need to be collected and analyzed.

3.1.4 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal is achieved through using standard techniques to collect and analyze representative samples, along with standardized data validation and reporting procedures. By following the guidance of this SAP, the goal of comparability between sampling events will be achieved. Historical water quality data from the survey area may be compared with data generated from this survey to enhance data analysis efforts. Previous data will be used if comparable sampling and analytical techniques were employed.

3.1.5 Sensitivity

The sensitivity of the methodology should be sufficient to indicate background levels of EDCs.

3.2 Sampling Stations and Selection Rationale

Sampling stations were chosen to provide the opportunity to characterize water concentrations of EDCs throughout the watershed. Samples will be collected from 7 stations with 2 stations located along the mainstem Green - Duwamish River and other stations on the major tributaries at or near the mouths of the major basins within the watershed, Black River/Springbrook Creek; Mill Creek; Soos Creek; and Newaukum Creek (Figure 2). Station names, locations, and coordinates, are shown below in Table 1.

3.3 Sampling Frequency

Samples will be collected during storm and baseflow conditions throughout the year 2003. The total number of sampling events of each type is up to 10 for storms events and four (quarterly) for baseflow events.

3.3.1 Storm sampling

Storm conditions are defined as ≥0.5 inches of precipitation in 12 hours. However, if few storms have been sampled as the wet season progresses, the precipitation criteria may be lowered in order to increase the number of samples collected. However, as stated above, sampling will target the rise and fall of the hydrograph; therefore, stream flows will have to be relatively stable prior to initiating sampling.

Table 1. Sampling stations for the Green - Duwamish Watershed EDC survey.

Sampling Station name	Site Id	Northing*	Easting*
Black River Pump Station	C317	176551.32	1291336.35
Green River (Fort Dent Park)	A310	173843.84	1290398.19
Springbrook Creek, near mouth	A317	173014.11	1294285.21
Mill Creek, near mouth	A315	137337.19	1289559.51
Soos Creek, above fish hatchery	A320	116829.74	1309993.27
Newaukum Creek, near mouth	0322	102374.40	1336714.40
Green River below HHD at USGS gaging station 12105900	E319	105097.71	1400560.26

*Coordinates are in Washington State plane feet - North; NAD83.

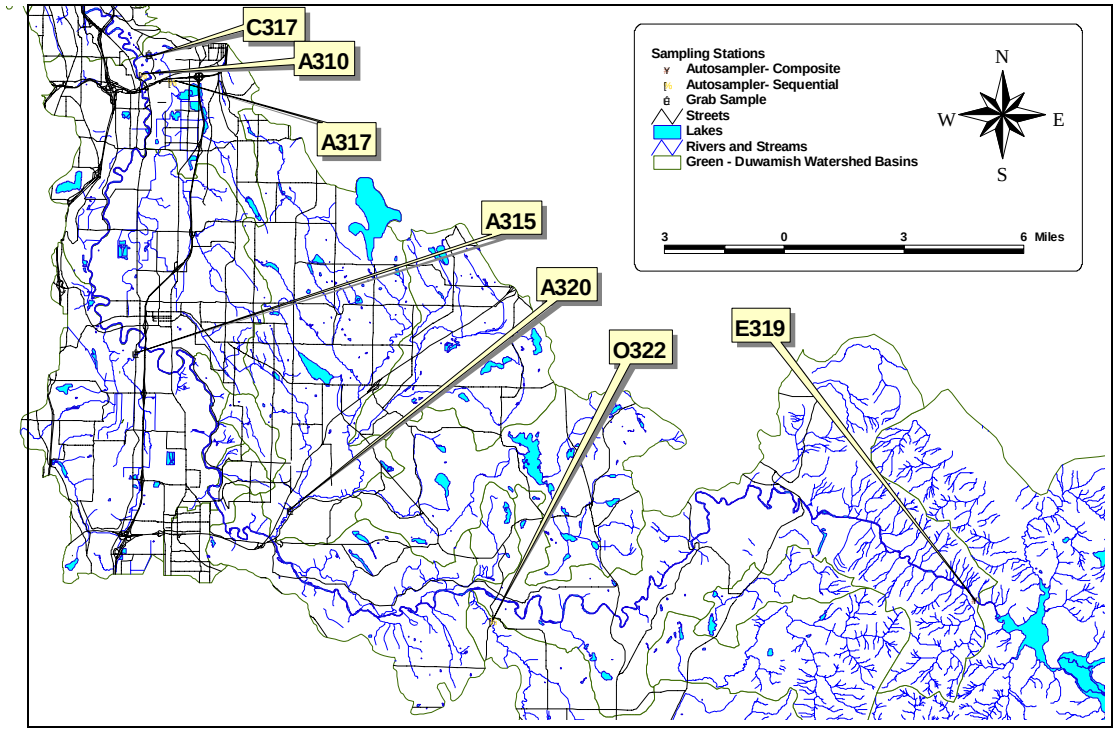

Figure 2. Sampling stations for GD watershed EDC survey.

3.3.2 Baseflow sampling

Baseflow conditions are non-storm conditions. Specific dry antecedent conditions are not required and baseflow samples will be collected quarterly.

3.4 Analytical Parameters

Analytical parameters for this survey were chosen based on literature reviews (Birkett and Lester, 2003; Davis et al, 1999; King County 2002a), concurrent with similar year-long surveys being preformed (Puget Sound EDC Survey and SWAMP EDC Survey), and analytical capabilities of the King County Environmental Laboratory.

Trace organic instrument analysis will include the following parameters:

- chlorinated pesticides;
- other pesticides (Atrazine and Vinclozolin)
- PAH compounds (both low (LPAH) and high (HPAH) molecular weight);
- phthalates;
- hormonal compounds;
- phenolics; and
- sewage tracers (caffeine, coprostanol, and dichlorobenzenes).

Immunoassay testing will be performed for two hormonal compounds, estradiol and ethinylestradiol.

The majority of analytical parameters for this survey were chosen due to their suspected endocrine disrupting potential. The complete list of analytical parameters includes some compounds not considered to be EDCs; however, this analytical suite was designed to encompass both this survey and other, concurrent King County surveys. Tables 2 and 3 summarize the target EDC and ancillary analytical parameters.

Table 2. Green - Duwamish Watershed EDC Survey Endocrine Disrupting Compounds (EDC) Parameter List.

EDC	Category	EDC	Category
Estradiol	Hormone	Aldrin	Pesticide
Estrone	Hormone	Atrazine	Pesticide
Ethinylestradiol	Hormone	Gamma-BHC (Lindane)	Pesticide
Methyltestosterone	Hormone	Alpha-Chlordane	Pesticide
Progesterone	Hormone	Gamma-Chlordane	Pesticide
Testosterone	Hormone	4,4'-DDE	Pesticide
Acenaphthene	LPAH	4,4'-DDT	Pesticide
Acenaphthylene	LPAH	Dieldrin	Pesticide
Anthracene	LPAH	Endosulfan I	Pesticide
Benzo(a)anthracene	HPAH	Endosulfan II	Pesticide
Benzo(a)pyrene	HPAH	Endrin	Pesticide
Benzo(b)fluoranthene	HPAH	Heptachlor	Pesticide
Benzo(k)fluoranthene	HPAH	Heptachlor Epoxide	Pesticide
Benzo(g,h,i)perylene	HPAH	Hexachlorobenzene	Pesticide
2-Chloronaphthalene	LPAH	Methoxychlor	Pesticide
Chrysene	HPAH	Vinclozolin	Pesticide
Dibenzo(a,h)anthracene	HPAH	Bisphenol A	Phenol
Fluoranthene	HPAH	2,4-Dichlorophenol	Phenol
Fluorene	LPAH	Pentachlorophenol	Phenol
Indeno(1,2,3-c,d)pyrene	HPAH	2,4,6-Trichlorophenol	Phenol
2-Methylnaphthalene	LPAH	Bis(2-ethylhexyl) Adipate	Plasticizer
Naphthalene	LPAH	Bis(2-ethylhexyl) Phthalate	Plasticizer
Phenanthrene	LPAH	Butyl Benzyl Phthalate	Plasticizer
Pyrene	HPAH	Diethyl Phthalate	Plasticizer
PCB Aroclors®	PCB	Di-n-butyl Phthalate	Plasticizer
Total 4-Nonylphenol	Surfactant		

Table 3. Green - Duwamish Watershed EDC Survey Ancillary Compounds Parameter List.

Ancillary Compound	Category	Ancillary Compound	Category
Alpha-BHC	Pesticide	1,2-Dichlorobenzene	Chlorobenzene
Beta-BHC	Pesticide	1,3-Dichlorobenzene	Chlorobenzene
Delta-BHC	Pesticide	Caffeine	Sewage Tracer
4,4'-DDD	Pesticide	1,4-Dichlorobenzene	Sewage Tracer
Endosulfan Sulfate	Pesticide	Carbazole	PAH Tracer
Endrin Aldehyde	Pesticide	Dibenzofuran	PAH Tracer
Toxaphene	Pesticide	Phenol	Phenol

4 SAMPLE COLLECTION AND HANDLING

The representativeness of a data set may be enhanced by following a standard set of protocols for collecting environmental samples. This section describes methodologies and protocols for the collection of representative water samples through the watershed, specifically for the analysis of trace organic compounds. All samples will be collected by subcontractors with coordination from the laboratory project manager of the King County Environmental Lab.

4.1 Sample Collection

The collection of samples for this survey will be done by the same set of proceedures detailed in the Green WQA Comprehensive Sampling and Analysis Plan (King County, 2002b). Below is overview of the techniques used in obtaining samples

4.1.1 Storm sample collection

The collection of storm samples will done by grab sample– at all stations. Grab samples will be collected while facing upstream to minimize contamination from field equipment. Whenever possible, the sampling should be conducted while facing the prevailing winds. See SOP # 02-02-13-000 (Clean Sampling using Surface Grabs)

4.1.2 Baseflow sample collection

The collection of baseflow samples will be done by grab sample collection at all stations. Grab samples will be collected while facing upstream to minimize contamination from field equipment. Whenever possible, the sampling should be conducted while facing the prevailing winds. See SOP # 02-02-13-000 (Clean Sampling using Surface Grabs)

4.2 Sample Handling

All samples will be kept in ice-filled coolers until delivery to the King County Environmental Laboratory. Upon receipt, all samples will be refrigerated to maintain a temperature of approximately 4°Celsius until analysis. All samples will be analyzed within method-specific holding times. Trace organic extractions will be completed within 7 days of sample collection and instrument analysis will be completed within 40 days of sample extraction. Immunoassay tests will be completed within 11 days of sample collection.

4.3 Field Quality Assurance/Quality Control

A strong field quality assurance/quality control (QA/QC) program that includes both standardized sampling protocols and the collection of field QC samples enhances the ability of the resulting data set to meet survey DQOs. The primary goal of the field sampling effort is to collect samples that are as free as possible of introduced contamination by target analytes. Several steps will be taken to minimize the potential for contamination and cross-contamination of samples.

Sampling personnel wear personal protective equipment that includes chemical-resistant gloves as part of King County's overall field safety program. The protective gloves can, however, potentially introduce phthalate compounds to samples that would be readily detectable during laboratory analysis. The King County Environment Laboratory has analyzed many types of

gloves for phthalate content. Sampling personnel for this project will wear chemical-resistant gloves that have the lowest potential for introducing phthalate compounds into the water samples.

4.3.1 Field Replicates

A field replicate is a second sample collected at a sampling station employing the same equipment and procedures used to obtain the first sample. The field replicate will be analyzed for same suite of analytes as the original sample. Analysis of field replicates is used to measure and document the repeatability of sample collection methodologies as well as provide data to assess environmental variability at the sampling station. One field replicate will be taken during each sampling event, either storm or baseflow.

5 TRACE ORGANIC LABORATORY ANALYSIS

The completeness and comparability of a data set is enhanced by following a standard set of protocols for analyzing samples. Analysis of a prescribed set of laboratory QC samples will also allow a data set to be evaluated in terms of precision, accuracy, and bias. This section describes trace organic analytical methodologies, associated QC protocols, and detection limits. The method detection limit (MDL) is that concentration at which an analyte can reliably be detected. The reporting detection limit (RDL) is that concentration at which an analyte can reliably be quantified. All detection limits are shown in units of micrograms per liter (µg/L).

5.1 Chlorinated Pesticides/PCBs

Chlorinated pesticide/PCB sample preparation will be performed according to EPA Method 3520C (SW 846 [EPA, 1986]), which is a continuous liquid-liquid extraction technique. About one liter of sample is extracted with approximately 400 ml of methylene chloride for 18 to 24 hours. The sample extract is split, for use in analysis of both chlorinated pesticides/PCBs and miscellaneous endocrine disrupting compounds (see Section 5.3). The chlorinated pesticide/PCB split is dried with sodium sulfate and concentrated to a 1-ml effective final volume. An alumina cleanup is performed on the split according to EPA Method 3610 (SW 846). Chlorinated pesticide/PCB sample analysis will be performed according to EPA Method 608. Table 4 lists the target chlorinated pesticide/PCB analytes and their respective detection limits.

Table 4. Target Chlorinated Pesticide/PCB Analytes and Detection Limits (µg/L).

Chlorinated Pesticide/PCB	MDL	RDL
Aldrin	0.005	0.01
Alpha-BHC	0.005	0.01
Beta-BHC	0.005	0.01
Delta-BHC	0.005	0.01
Gamma-BHC (Lindane)	0.005	0.01
Alpha-Chlordane	0.005	0.01
Gamma-Chlordane	0.005	0.01
4,4'-DDD	0.005	0.01
4,4'-DDE	0.005	0.01
4,4'-DDT	0.005	0.01
Dieldrin	0.005	0.01
Endosulfan I	0.005	0.01
Endosulfan II	0.005	0.01
Endosulfan Sulfate	0.005	0.01
Endrin	0.005	0.01
Endrin Aldehyde	0.005	0.01
Heptachlor	0.005	0.01
Heptachlor Epoxide	0.005	0.01
Methoxychlor	0.025	0.05
Toxaphene	0.050	0.10
PCB Aroclors®	0.050	0.10

5.2 Base/Neutral/Acid Extractable Semivolatile Compounds (BNAs) and Atrazine

Sample preparation for BNAs, which will include Atrazine (a triazine pesticide), will be performed according to EPA Method 3520C, described in Section 5.1. The extraction will be performed on a separate 1-liter volume of sample matrix; however, no sample cleanup will be necessary. BNA/Atrazine sample analysis will be performed according to EPA Method 8270C (SW846), which uses gas chromatography with mass spectroscopy (GC-MS), retrofitted with a large volume injector (LVI) to lower the detection limits. Table 5 lists the target BNA/Atrazine analytes and their respective detection limits.

Table 5. Target BNA Analytes (including Atrazine) and Detection Limits (µg/L).

BNA Compound	MDL	RDL
Acenaphthene	0.010	0.050
Acenaphthylene	0.010	0.050
Anthracene	0.010	0.050
Atrazine (Triazine Pesticide)	0.050	0.100
Benzo(a)anthracene	0.025	0.050
Benzo(a)pyrene	0.010	0.025
Benzo(b)fluoranthene	0.010	0.025
Benzo(g,h,i)perylene	0.100	0.250
Benzo(k)fluoranthene	0.010	0.025
Benzyl Butyl Phthalate	0.010	0.025
Bis(2-Ethylhexyl)Phthalate	0.010	0.025
Caffeine	0.025	0.050
Carbazole	0.025	0.050
2-Chloronaphthalene	0.010	0.050
Chrysene	0.025	0.050
Dibenzo(a,h)anthracene	0.100	0.250
Dibenzofuran	0.010	0.025
1,2-Dichlorobenzene	0.050	0.250
1,3-Dichlorobenzene	0.050	0.250
1,4-Dichlorobenzene	0.050	0.250
2,4-Dichlorophenol	0.500	1.000
Diethyl Phthalate	0.010	0.025
Di-N-Butyl Phthalate	0.010	0.025
Fluoranthene	0.010	0.025
Fluorene	0.010	0.025
Hexachlorobenzene	0.025	0.050
Indeno(1,2,3-Cd)Pyrene	0.100	0.250
2-Methylnaphthalene	0.100	0.500
Naphthalene	0.025	0.050
Pentachlorophenol	1.000	2.000
Phenanthrene	0.010	0.025
Phenol	0.500	1.000
Pyrene	0.010	0.025
2,4,6-Trichlorophenol	0.500	1.000

5.3 Miscellaneous Endocrine Disrupting Compounds

The "miscellaneous endocrine disrupting compounds" include a pesticide (Vinclozolin), three BNA compounds, and six hormones (see Table 6). Sample preparation for these analytes will be performed as described in Section 5.1. After splitting the extract, the miscellaneous endocrine disrupting compound split will be water-washed as a cleanup procedure. Sample analysis for these compounds will be performed by GC-MS with LVI, operated in the Selected Ion Monitoring (SIM) mode. Table 6 lists the target miscellaneous endocrine disrupting compounds and their respective detection limits.

Table 6. Miscellaneous Endocrine Disrupting Compounds and Detection Limits (µg/L).

ED Compound	MDL	RDL
Bis(2-ethylhexyl)adipate**	0.010	0.100
Bisphenol A**	0.010	0.100
Estradiol***	0.010	0.025
Estrone***	0.010	0.025
Ethynyl estradiol***	0.010	0.025
Methyltestosterone***	0.010	0.025
4-Nonylphenol (total)**	0.020	0.100
Progesterone***	0.010	0.025
Testosterone***	0.010	0.025
Vinclozolin*	0.010	0.025

* Pesticide
** BNA Compound
*** Hormone

5.4 Laboratory Quality Control

Trace organic laboratory QC samples will include method blanks, spike blanks, matrix spikes, matrix spike duplicates, and surrogates. Method blanks and spike blanks will be analyzed at a frequency of one per analytical batch. Matrix spikes, and matrix spike duplicates will be analyzed at a frequency of one per analytical batch or a minimum of one per 20 analytical samples extracted within 14 days. Surrogates are analyzed with every QC and analytical sample. Table 7 summarizes the control limits for trace organic laboratory QC samples.

5.4.1 Method blank

A method blank is an aliquot of a clean reference matrix, such as deionized, distilled water for water samples, which is processed through the entire analytical procedure. Analysis of method blanks is used to evaluate the levels of contamination that might be associated with the processing and analysis of samples. Method blank results should be "less than the MDL" for all target analytes.

5.4.2 Spike blank

A spike blank is an aliquot of clean reference matrix, such as deionized distilled water for water samples, to which a known concentration of one or more target analytes has been added. The spiked aliquot is processed through the entire analytical procedure. Analysis of the spike blank is used as an indicator of method performance and can also be used in conjunction with matrix spike results as an indicator of sample matrix effects. Control limits are based on the percent recovery of the spiked compounds.

5.4.3 Matrix spike

A matrix spike (MS) is a known concentration of one or more target analytes, which is introduced into a second aliquot from one analytical sample. The spiked sample is processed through the entire analytical procedure. Analysis of the MS is used as an indicator of sample matrix effect on the recovery of target analytes. Control limits are based on the percent recovery of the spiked compounds.

5.4.4 Matrix spike duplicate

A matrix spike duplicate (MSD) is a known concentration (same as the MS) of target analytes, which is introduced into a third aliquot of the same analytical sample. The spiked sample is processed through the entire analytical procedure. Analysis of the MSD is used as an indicator of sample matrix effect on the recovery of target analytes as well as method precision. The relative percent difference (RPD) between the MS and MSD results is calculated; however, control limits are not maintained. The RPD for MS/MSD results is, instead, reviewed during the data validation and analysis process to evaluate any data quality issues arising from questions of analytical precision.

5.4.5 Surrogate

A surrogate is a known concentration of one or more non-target analytes which is added to every sample (both analytical and QC samples) prior to extraction. Analysis of surrogates is used as an indication of method or matrix bias for target compounds on a sample-specific basis. Surrogate compounds are selected that behave in a similar manner to target analytes. Control limits are based on the percent recovery of the surrogate compounds.

Table 7. Trace Organic Laboratory QC Samples and Control Limits.

QC Sample	Chlorinated Pesticides and PCBs	BNAs/Atrazine	Misc. Endocrine Disrupting Comp.
Method Blank Result	All compounds <MDL	All compounds <MDL	All compounds <MDL
Spike Blank Recovery	23 to 139%*	9 to 127%*	50 to 150%
MS/MSD Recovery	23 to 139%*	9 to 127%*	50 to 150%
MS/MSD RPD	Not Applicable	Not Applicable	Not Applicable
Surrogate Recovery	50 to 150%	10 to 141%*	50 to 150%

*Low to high range of all compounds used for surrogates or spikes. Control limits for individual compounds are equivalent to the current lab acceptance limits.

QC sample results that exceed control limits will be evaluated to determine appropriate corrective actions. Samples will typically be reanalyzed if unacceptable QC results indicate a systematic problem with the overall analysis, and if sufficient sample matrix is remaining and the analytical holding time has not expired. Unacceptable QC results caused by a particular sample or matrix will not require reanalysis unless an allowed method modification would improve the results. Analytical results that are outside of QC control limits will be qualified and flagged according to procedures outlined in Section 7.

6 Immunoassay Testing for Estradiol and Ethinylestradiol

Enzyme-linked immunosorbent assay (ELISA) testing will be performed on water samples to measure concentrations of two hormones, estradiol and ethinylestradiol. Trace organic GC-MS results will be used to evaluate results from the ELISA testing and to assess the potential of using these relatively low-cost, rapid assays as a reliable tool for measuring concentrations of hormonal compounds in natural waters.

Data comparability between GC-MS and ELISA results will be evaluated by the data comparability guidelines established in EPA (1996). Data comparability analysis will include development of statistical correlation between GC-MS and ELISA results. Development of statistically-valid correlation factors will be dependent on having a sufficient number of results greater than the GC/MS MDL in the data set.

6.1 Estradiol

The quantitative analysis of estradiol (17b-estradiol) in water samples will employ the American Laboratory Products (ALPCO) Estradiol Plate Kit®. This estradiol ELISA kit is based on the competition principal in which an unknown amount of estradiol present in the sample and a fixed amount of estradiol conjugated with horse-radish peroxidase (HRP) compete for a fixed number of binding sites to polyclonal estradiol antiserum coated onto microtiter wells.

After a two-hour incubation, the microtiter plate is washed to remove the unbound HRP conjugate. A substrate is then added and the plate incubated for 15 minutes. The enzyme-substrate reaction is stopped with acid and the color that has developed in the wells is measured in a colorimeter at 450 nanometers (nm). The color measurement is proportional to the bound enzyme conjugate and inversely proportional to the estradiol concentration in the water sample.

This method measures the concentration of free, unconjugated estradiol in natural water samples. The estradiol ELISA test has a reported MDL of 0.020 µg/L in both fresh and salt water. Samples may be concentrated, using EPA Method 3535A (SW846) Solid-Phase Extraction (SPE) technique, to detect low-level ambient concentrations of estradiol. The maximum concentration that can be measured without dilution is 0.50 µg/L, which is the highest standard on the calibration curve.

6.2 Ethinylestradiol

The qualitative analysis of ethinylestradiol in water samples will employ the Ridascreen Ethinylestradiol Plate Kit®. This ethinylestradiol ELISA kit uses a double antibody system. The anti-ethinylestradiol antibodies are added to the wells together with the ethinylestradiol-enzyme conjugate and the test sample. The anti-ethinylestradiol antibodies bind to a fixed number of immobilized sheep antibodies in the wells. A fixed amount of ethinylestradiol-enzyme conjugate and the unknown amount of ethinylestradiol in the sample compete for the binding sites on the anti-ethinylestradiol antibodies.

After a two-hour incubation, the microtiter plate is washed to remove the unbound conjugate. A substrate and chromogen are then added and the plate is incubated for 30 minutes. Bound enzyme conjugate converts the colorless chromogen into a blue product. The enzyme-substrate reaction is stopped with acid which leads to a color change from blue to yellow. The color that

has developed in the wells is measured in a colorimeter at 450 nm. The color measurement is inversely proportional to the ethinylestradiol concentration in the water sample.

This method measures the concentration of free, unconjugated ethinylestradiol in natural water samples. The ethinylestradiol ELISA test has a reported MDL of 0.030 μg/L in both fresh and salt water. Samples may be concentrated, using SPE, to detect low-level ambient concentrations of ethinylestradiol. SPE concentration factors will be adjusted to bring measurements within the range of standards. The concentration factors applied will be reported with each set of sample results. The maximum concentration that can be measured without dilution is that of the highest standard, 1.08 μg/L.

6.3 Immunoassay Testing QC Procedures

The following QC procedures will be used for both estradiol and ethinylestradiol methods. The particular QC samples analyzed will depend on whether the SPE technique is utilized for a particular batch of samples.

- A **method blank** is an aliquot of a clean reference matrix, which is processed through the entire analytical procedure when the SPE technique is used. Analysis of method blanks is used to evaluate the levels of positive bias that might be associated with the processing and analysis of samples. Method blank results should be “less than the MDL” for each target analyte.
- A **negative control** is included with each ELISA kit and is analyzed in duplicate with each batch of samples. The negative control is not processed through the SPE technique and is equivalent to a method blank for samples where the SPE technique is not used. Negative control results should be "less than the MDL" for each target analyte.
- A **spike blank** is an aliquot of clean reference matrix, to which a known concentration of the target analyte has been added. The spiked aliquot is processed through the entire analytical procedure, when the SPE technique is used. Analysis of the spike blank is used as an indicator of method performance and can be used in conjunction with matrix spike results as an indicator of sample matrix effects. Control limits are based on the percent recovery of the spiked compounds.
- A **matrix spike (MS)** is a known concentration of one or more target analytes, which is introduced into a second aliquot from one analytical sample. The spiked sample is processed through the entire analytical procedure when the SPE technique is used. Analysis of the MS is used as an indicator of sample matrix effect on the recovery of target analytes. Control limits are based on the percent recovery of the spiked compounds.
- A **positive control** is a separate portion of the mid-point calibration standard that is analyzed in duplicate with each batch of samples. The positive control is not processed through the SPE technique. Both the percent recovery of the positive control and the difference between the duplicate measurements are evaluated.

7 DATA VALIDATION, REPORTING, AND RECORDKEEPING

Data validation is critical for evaluating how well analytical data meet project DQOs. Data validation is performed, at some level, during several steps in the process of sample analysis. All trace organic instrument analytical data will be entered into King County's Laboratory Information Management System (LIMS).

7.1 Data Validation

Laboratory analytical data are reviewed, first by the primary analyst and then by a peer reviewer. Analytical data are reviewed for completeness and QC sample data are reviewed for compliance with project and method QA/QC requirements. If there are any QC failures at this point, corrective action may be taken or qualifier flags applied to the data.

A laboratory project manager (LPM) will provide the next data review step, at a project level. The LPM will verify the completeness of an entire data set and report any QC failures or anomalies. A project data validator will provide a final review of the data to ensure they meet the project DQOs. Data then will be reported in a variety of formats, depending on project needs.

7.2 Data Reporting

Immunoassay results will be reported as quarterly narrative reports of results and associated QC testing. Sample and QC results will be submitted to the LPM and data validator as electronic files in Excel® format until such time that the data are accessible through LIMS. At present, the necessary programming and software testing required to make immunoassay data accessible through LIMS is expected to be completed in early 2004.

All laboratory analytical data are maintained in perpetuity on LIMS. Data may be viewed on-line in LIMS by King County personnel only. Project data may also be downloaded from LIMS into a hard copy format using Microsoft Excel®. Analytical data will be reported on a routine basis in Excel® format along with an accompanying QA/QC review narrative.

Laboratory analytical data may be stored with data qualifier flags indicating QC failures. The flag "B" is used to indicate possible laboratory contamination of a sample and is applied when a target analyte is detected in the laboratory method blank. Sample results that are above the MDL but that are less than ten times the concentration detected in the method blank will be qualified with a "B" flag. The flag "H" is used to indicate a sample handling condition that did not meet method requirements. Handling conditions may include an improper sample container, improper preservation of the sample, or an excedence of the method-specific holding time. The flag "E" may be applied to sample data at the discretion of the laboratory analyst or peer reviewer, should control limits on one or more QC samples not be met. The flag "E" indicates that sample data should be viewed as estimated.

Analytical results from field replicates will be reviewed to evaluate their impact on the quality and usability of sample analytical data. Results from field QC samples will not be used to flag sample analytical data but will be taken into consideration during final data review, analysis, and reporting.

7.3 Recordkeeping

Hard-copy field notes, raw analytical data, and any other hard-copy project information will be stored according to standard King County Environmental Laboratory practices for a period of ten years.

7.4 Special Data Qualification for BNA Compounds and Atrazine

Every reported compound will be evaluated with a continuing calibration standard for each 12-hour shift. The acceptable continuing calibration percent difference is 80 to120% for every target analyte. Sample data for any detected target analyte for which the percent recovery is greater than 120% will be qualified with an "L" flag. Sample data for any detected target analyte for which the percent recovery is less than 80% but equal to or greater than 50% will be qualified with a "G" flag. If the percent recovery for any target analyte is less than 50%, either corrective action will be taken to meet the 50% criterion and the samples rerun or associated sample data for the target analyte will be qualified with a "G" flag.

These qualifier flags indicate that:

- L – the reported value may be biased high, based on continuing calibration information;
- G – the reported value may be biased low, based on continuing calibration information;

Any target analyte reported at a concentration between the MDL and RDL will be qualified "<RDL." If this value is less than the lowest concentration in the calibration curve, the sample data will also be qualified with an "E" flag, indicating that the reported concentration is estimated.

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