Randall Marshall, WET Coordinator
(360-407-6445 or rmar461@ecy.wa.gov)
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2010 Pilot of an Integrated Ambient Monitoring Approach for Urban Streams
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Introduction

Purpose

We will demonstrate in spring 2010 a method for assessing the suitability of streams for supporting salmon early lifestages and the food they need to survive and grow. Successful salmon reproduction is the most important feature of a healthy stream in the Pacific Northwest. Protecting early lifestages of salmon and the food upon which they depend is the key to maintaining productive streams. Doing so will tend to protect other fish and wildlife as well. The demonstration will include in situ toxicity testing with rainbow trout and daphnids and bioassessments of benthic invertebrates and periphyton. Passive samplers for metals, polar organics, and nonpolar organics will generate lists of candidate stressors and gene microarrays applied to the fry and daphnids after in situ exposure will provide indications of initial responses to those chemical stressors.

A successful demonstration will give us a candidate monitoring approach based upon biological responses with at least a minimal ability to identify stressors causing adverse effects. The pilot is designed to answer questions about the utility of the technologies themselves. The most important of the questions is whether the technologies can be integrated so that information generated concurrently will provide a diagnosis of the sources of biological impairment. Even if the diagnosis is rough, it is far better than dead test organisms or missing benthic taxa and no explanation to offer the public or guide future work. These techniques can then be developed for an economy of scale and the field work can ultimately be performed by trained city employees or volunteers.

Background

The Clean Water Act's objective is to restore and maintain the chemical, physical, and biological integrity of the nation's waters. For more than forty years, efforts to achieve this objective have focused on controlling municipal and industrial wastewater discharges. Discharge monitoring has driven the implementation of these controls. However, discharge monitoring does not assess the integrity of a water body. Discharge monitoring only provides a rough estimate of potential environmental effects based upon limited information on pollutant concentrations over time in relationship to variable receiving stream chemistry and flow. Storm water discharge monitoring will generate information that is certain to be inadequate for evaluating urban stream health given the large number of storm water outfalls discharging highly variable volumes containing rapidly changing pollutant concentrations. Regularly monitoring all storm water outfalls for every potential pollutant would be very expensive and generate information of limited usefulness in assessing stream health. In addition, the detection of unknown or illegal discharges is too often left to chance resulting in a possibly serious information gap when considering stream health.

Test organisms placed in a stream (in situ toxicity testing) would both experience a realistic environmental exposure and be able to respond to a broad spectrum of toxic chemicals. Passive samplers deployed alongside the test organisms would accumulate chemicals in a manner similar to living organisms and could be analyzed for metals, polar organics, and nonpolar organics (**Table 4**). Measuring test organism responses at the molecular level using gene microarrays would enhance knowledge of the effects on the in situ test organisms of any chemicals detected and could provide an alert that chemicals need to be added to the analysis list. In situ toxicity testing, gene microarrays applied to the test organisms, and monitoring with passive samplers all have potential for an economy of scale if used routinely. See **Tables 2** and **3,** and **Figure 1** for a summary of study activities, locations, and participants.

Potential Regulatory Application

The most important steps for controlling damage to streams from storm water consist of reducing discharge volumes, eliminating surge flows, removing suspended solids, and controlling sources of the metals, pesticides, and polycyclic aromatic hydrocarbons (PAHs) not eliminated by solids removal. Until these steps have been completed, monitoring to assess water quality is only a distraction from efforts to achieve good water quality. Once these steps to reduce storm water impacts have been completed, the streams themselves should be monitored to determine the adequacy of all water pollution control efforts. Monitoring receiving waters might also discover other pollutant sources that we have so far missed.

If the in situ test organisms show adverse effects, then the microarray and passive sampling results might give an indication of which chemicals need more scrutiny. If confidence in the initial results is high enough, then a search for sources of the candidate pollutants makes sense. If initial results yield no candidate toxicants, then the ambient monitoring might be repeated upstream in order to get closer to sources and have stronger effects or downstream in order to have more in situ test organisms left alive at the end and available for microarray exposure. If the in situ test organisms show no adverse effects and the benthic community appears healthy, then another location should be selected for ambient monitoring.

Experience might eventually lead to regulatory responses based solely on microarray results, but not until we have established criteria and developed appropriate responses. The information accumulated from the microarrays and passive samplers might reveal new chemicals of concern. For example, the trout microarray might respond to environmental estrogens or immune system compromising chemicals and makes us aware of a potential problem that in situ testing did not reveal.

Monitoring of receiving waters already has a start in our state. The SeaTac Airport storm water permit requires testing stream samples for toxicity to rainbow trout embryos and has withstood appeal before the Pollution

Control Hearings Board. The Port of Seattle (permit holder for the airport) is currently planning to do in situ trout testing as a substitute for the lab testing. Pierce County successfully conducted a study using in situ trout testing in a few urban streams in spring 2008. If the pilot is successful and an economy of scale can be established to make the approach affordable, we could implement instream monitoring to assess the adequacy of current pollutant controls and protect salmon reproduction in urban streams. We might also discover pollution sources that we missed along the way. This approach could help guide us to the watershed management envisioned by the National Research Council in its EPA-sponsored 2008 report on *Urban Stormwater Management in the United States* where regulatory responsibility is centered at the local level with state oversight, and all permittees share the cost for monitoring watershed health.

Project Description

Monitoring Locations

Indian Creek in downtown Olympia is known by the Thurston County Water and Waste Management Stream Team to be adversely impacted by urban pollutants. Indian Creek originates from Bigelow Lake, which has sediments with elevated metals concentrations, and then flows within various ditches and pipes through an urbanized and industrialized area. The Benthic Invertebrate Index of Biological Integrity (BIBI) shows the area near our downstream monitoring station to have only moderate biological integrity. The City of Olympia is working to improve fish passage and habitat in Indian Creek. The creek supports small salmon runs. The Indian Creek work will include upstream and downstream stations.

Table 1 Monitoring Stream

Study Stream Descriptions	**Basin Characteristics**				**Population Forecasts**			
	acres	urban	impervious	forested	2010	per acre	2030	per acre
Indian Creek	1,500	29.3%	22.3%	20.4%	5,250	3.50	6,530	4.35

Sources of data:
Thurston Regional Planning Council. 2001. Land Cover Mapping of Thurston County.
Thurston Regional Planning Council. 2003. The relationship of Land Cover to Total and Effective Impervious Area.
Thurston Regional Planning Council. 2007. Population and Employment Forecast Work Program.

Monitoring Details

Toxicity Tests and Bioassessments

Environment Canada developed toxicity tests using the embryo, alevin, and fry lifestages of rainbow trout (*Oncorhynchus mykiss*) because of concern over water quality in salmon spawning streams. Each lifestage is sensitive to different pollutants. A test on all of these lifestages combined is a true chronic test. The biological effects assessed by this testing include mortality, failure to hatch, abnormal development, and reduced growth. A trout egg through fry test works well either in the lab or in a hatch box enclosed in a wire basket full of rocks exposed in a stream. We will conduct in situ testing at upstream and downstream Indian Creek stations with a control exposed to clean water in a lab.

Thurston County and Washington State University (WSU) will conduct in situ testing with *Daphnia magna*. Daphnids are among the most common toxicity test organisms in the world because of their reliability and sensitivity. Because of their popularity in toxicity tests, the database of daphnid responses to individual chemicals is quite large. Because they are related to many of the benthic invertebrates, daphnid responses in toxicity tests also provide information relevant to benthic invertebrate assessments.

In order to assess effects on the insects and crustaceans important as food for salmon fry and juveniles, we will conduct instream benthic invertebrate and periphyton assessments. Invertebrates are more sensitive than fish to many pollutants such as metals and insecticides. Benthic invertebrate assessments are now standard tools for determining stream health. The replacement of pollutant sensitive species with pollutant tolerant species is easily measured. We will also examine the invertebrates which colonize clean rocks placed in extra wire baskets and deployed for 28 days at the monitoring stations. If the colonization results prove useful, we will have a technique to supplement standard instream bioassessments of benthic invertebrates especially in deeper streams or other difficult circumstances.

Gene Microarrays

Gene microarray analysis measures the expression of hundreds or thousands of genes from an organism which has been exposed to chemical pollutants. Microarrays for assessing environmental contaminants evolved from microarrays used to study developmental processes or basic physiology. Microarrays note when genes are turned on and when they are turned off. A gene might turn on to resist toxicity or turn off because of interference from a chemical. Scientists at the University of California at Berkeley have discovered patterns of microarray response that are diagnostic of copper exposure. The manufacture and reading of microarrays have been automated. Some tissue preparation is needed prior to application to the microarray. An economy of scale is possible and the amount of information which can be gained on chemical effects is large.

Environment Canada developed a rainbow trout gene microarray which will be used by the United States Geological Survey (USGS) on the fish exposed at our stream stations and on a lab control. In addition, we will expose trout in a lab to a sample of primary effluent diluted to just below the threshold for lethality so that the trout microarray can be run for comparison on both whole fish and livers only. Because the fry are so small, using whole fish will save time and money and may allow better assessment of toxicant effects on growth and development. On the other hand, the liver is the site of many known responses to toxicity and using whole fish might raise detection limits too much for the liver responses. Our comparison should shed some light on whether whole fish or livers work best in microarrays.

The University of California (UC) at Berkeley has developed a *Daphnia magna* gene microarray and will conduct the microarray on the daphnids from the in situ exposures. They will also conduct the microarray on daphnids exposed in a lab to a downstream sample at 12° and 25° C in order to assess differences in gene expression relative to temperature. Previous daphnid microarray work at UC has involved daphnids exposed at 20° - 27° C. Daphnid microarrays are run on whole organisms.

Biomarkers

A biomarker is a chemical produced in a living organism in response to toxicity. A gene on a microarray which is turned on by chemical exposure is usually the gene which produces the biomarker. Biomarkers include enzymes produced to fight toxicity or enzymes with another purpose whose production is affected by toxic chemicals. Each biomarker responds to specific types of chemical and can be a valuable diagnostic tool. Biomarker response is longer lived than microarray responses and can provide useful information for some time after chemical exposure. If metallothionein is induced in an organism for example, its presence is an indication that the organism was exposed to metals at concentrations and conditions sufficient to produce toxicity.

These biomarkers will be used in the pilot:

> Metallothionein is the enzyme produced by an organism in response to exposure to a toxic metal.
> Vitellogenin is a protein produced when an organism is exposed to an endocrine disruptor resembling estrogen. The protein is normally only produced in females during egg production.

Passive Samplers

We will deploy passive samplers for organic chemicals and metals along with the in situ test chambers. Passive samplers serve the same purpose as a composite sampler for characterizing average chemical exposure over a time period but can be deployed for a longer time with a level of effort similar to collecting a grab sample. In addition, a passive sampler is coated with a substance that absorbs pollutants in way similar to living organisms and provides a better exposure assessment than provided by a chemical analysis of a grab or 24-hour composite water sample. Results of the analysis of passive samplers will help interpret toxicity test and microarray results. By using passive samplers for metals, polar organics (water soluble compounds), and nonpolar organics (fat soluble compounds), the study will cover many pollutants of concern for wastewater and runoff. See **Table 4** for a detailed list of analytes. The passive samplers are:

- DGT and SLMD for metals including cadmium, copper, nickel, lead, and zinc.
- POCIS for polar organics including herbicides, nonylphenol, and carbamate pesticides.
- SPMD for nonpolar organics including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, organophosphate pesticides, nitrogen pesticides, and other organic chemicals.
- Baskets of rock for assessing benthic invertebrate colonization which can potentially provide simplicity, flexibility, and an economy of scale for benthic invertebrate assessments.

Special Metals Focus

Because metals are common storm water pollutants of concern, the pilot will explore in detail instream metals chemistry and effects.

- We will compare the results from the 2 types of passive samplers (SLMDs and DGTs) for metals in order to provide comparisons that might show the newer SLMDs to be preferable to the better established DGTs which are more expensive and must be deployed for shorter durations.
- We will collect grab samples from Indian Creek three times at each station to analyze for the same metals to be measured in the passive samplers. Measuring water concentrations of the metals will help evaluate passive sampler results in general and perhaps shed light on the comparisons of the two types of metals sampler.
- We will also have the samples analyzed for the biotic ligand model (BLM) parameters and run the BLM for copper. The BLM predicts metals toxicity based upon competition for gill binding sites. The copper BLM is generally accepted and recommended by EPA for determining water quality criteria for copper. The BLM does not work as well at predicting toxicity from other metals, but the same chemical principles apply and the copper BLM will reveal the tendencies for the other metals. The BLM might also shed light on SLMD and DGT performance.
- We will test trout tissue from the in situ exposures for metallothionein, an enzyme produced as a defense mechanism for toxic chemical exposure.
- We will test trout tissue from the in situ exposures for metals concentrations. Tissue metals concentrations can be used to evaluate which passive sampler gives the most meaningful results.
- The daphnid microarray might also respond to metals. The scientists doing the daphnid microarray have shown the ability to use gene responses in the microarray as an indicator of copper exposure.

Other Specific Questions Explored in the Project

The discussion below focuses on major questions of interest other than metals. **Table 2** lists all of the comparisons which will be performed on data generated by the study.

- Fish microarrays are usually run on liver tissue because the liver is the site of many biochemical responses to toxic chemicals. However, toxic chemicals also affect genes which are expressed in the whole fish such as those for growth or development. However, running microarrays on whole fish in

order to assess the broad range of responses dilutes the liver responses and reduces the ability to measure them. On the other hand, the livers in the trout fry are so small that extracting sufficient liver tissue for microarray requires many fish and is very expensive. We will run trout microarrays both on whole fish and extracted livers to assess the differences in response to a primary-treated municipal effluent and help resolve the dilemma.

- The UC Berkeley scientists used daphnids exposed at 20° - 27° C in daphnid microarray development. Indian Creek will be around 10° to 12° C during the in situ exposures. In order to assess differences in gene expression between daphnids exposed at 20° - 27° C and ambient stream temperatures, we will expose daphnids Indian Creek samples in a lab at 12° C and 25° C and then run microarrays.
- The endocrine disruptor chemicals (EDCs) of most concern are the ones with estrogenic activity. We need to determine the degree to which very young trout fry respond to environmental estrogens. They may not respond the same as older fish. We will expose trout to estradiol in a lab and measure vitellogenin for comparison to known responses of older fish.
- How convenient and useful are passive samplers, gene microarrays, and biomarkers?
- In order to assess effects on the insects and crustaceans important as food for salmon fry and juveniles, we will conduct instream benthic invertebrate and periphyton assessments. The replacement of pollutant sensitive species with pollutant tolerant species is easily measured. We will also examine the invertebrates which colonize clean rocks placed in extra wire baskets and deployed for 28 days at the monitoring stations. If the colonization results prove useful, we will have a technique to supplement standard instream bioassessments especially in deeper streams or other difficult circumstances.
- A biomarker is a chemical produced in a living organism in response to toxicity. A gene on a microarray which is turned on by chemical exposure is usually the gene which produces the biomarker. Biomarker response is longer lived than microarray responses and can provide useful information for some time after chemical exposure. We will compare the metallothionein response to the microarray response to see if the biomarker's persistence confers an advantage over microarrays.

Possible Future Developments

- Reduce the number of genes on the microarrays to those shown by experience to be the most useful and meaningful. This will improve detection and precision and reduce the cost. Genes or patterns of genes which relate to test organism response or to elevated chemical concentrations in the passive samplers qualify as useful.
- Try the trout microarray on other fish species held in situ or on feral fish.
- Get a microarray reader for the King County Environmental Lab (KCEL) for use in routine monitoring. KCEL can and does do testing for other local governments. They also have many environmental questions of their own such as Coho prespawn mortalities and the effects of EDCs.
- Expose trout or daphnids in a lab to extracts from the passive samplers to try to find the same pattern of gene expression found in the in situ test organisms. This could narrow the list of candidate toxicants and aid in identifying sources.
- Use older trout fry for shorter in situ exposures to assess effects on more active fish. Active trout fry require food which may not drift into the in situ chambers and we must limit exposure time.
- Consider using a more complete set of biomarkers. Biomarkers have an advantage over microarrays because some biomarkers can persist for awhile and the mRNA assessed by a gene microarray lasts somewhere on the order of a day before breaking down. If a cost-effective approach to biomarker measurement can be found, it would be worth pursuing. Other potentially useful biomarkers include:
    - Cytochrome p450 (CYP1A/EROD) is the enzyme produced by organisms in response to an exposure to a toxic nonpolar organic chemical in order to catalyze its breakdown and removal.
    - Acetylcholinesterase is the enzyme inhibited by organophosphorus or carbamate pesticides.
    - Superoxide dismutase (SOD) is the enzyme produced by an organism in response to exposure to an oxidizing chemical.

Table 2 Data Comparisons in Project

Comparisons in Integrated Ambient Monitoring Pilot Project at Indian Creek

Actions	Comparisons
Instream Biological Assessments	
benthic invertebrate bioassessment	upstream vs. downstream & to all chemistry (passive sampler and water) and daphnid results to look for potential causes
periphyton assessment	upstream vs. downstream & to chemistry (passive sampler and water) results to look for potential causes
trout in situ toxicity testing	upstream vs. downstream & to passive sampler and microarray results to look for potential causes
trout gene microarray	upstream vs. downstream & to all passive sampler, water chemistry, and biomarker results to look for potential causes
fish tissue metals	to see how much agreement with DGT and SLMD results
trout metallothionein	to see if DGT, SLMD, water chemistry, and tissue metals are predictive
daphnid in situ toxicity testing	upstream vs. downstream & to passive sampler, microarray and BLM results to look for potential causes + help interpret bioassessments
daphnid gene microarray	upstream vs. downstream & to passive sampler and other chemistry to look for potential causes + help interpret bioassessments
Laboratory Biological Assessments	
trout gene microarrays on whole fish and on liver from fish exposed in a lab to a contaminated sample	whole fish vs. liver only to determine differences in gene expression and whether whole fish are the best alternative
trout vitellogenin on trout exposed in a lab to estradiol	lab control vs. estradiol treatment to determine the extent to which very young fry respond to EDCs based upon known responses from databases and literature; could also be helpful in interpreting microarray results
microarray on daphnids exposed in a lab to downstream samples at 12°and 25°C	12°C vs. 25°C to determine the degree to which in situ results reflect the UC-Berkeley microarray experience on daphnids exposed at 20° - 27°C
Passive Samplers and Analysis	
metals analysis - (DGT and SLMD) for **Cd**, **Cu**, **Ni**, **Pb**, and **Zn**	DGT vs. SLMD to see which works best & comparison to bioassessment, in situ test, and microarray results to look for potential causes
analysis SPMD - **BNAs** (including **PAHs**) and **pesticides (Cl,OP,N)**	comparison to invertebrate and periphyton bioassessment, trout and daphnid in situ, and microarray results to look for potential causes
passive sampler polar - POCIS analyzed for **herbicides**, **pesticides (Cl,OP,N)**, **carbamates**, and **BNAs**.	comparison to invertebrate and periphyton bioassessment, trout and daphnid in situ, and microarray results to look for potential causes
Water Chemistry	
3 stream grab sample metals analyzed for **Cd**, **Cu**, **Ni**, **Pb**, and **Zn**	reality check for DGT and SLMD results & supplement for interpreting in situ toxicity test, lab toxicity test, and microarray results
Biotic Ligand Model (BLM) based upon **Cu** and - **DOC, pH, Ca, Mg, Na, K, SO_4, Cl, and alkalinity**	interpretation of in situ, microarray, and trout metallothionein results + potentially useful in understanding the role of ion competition for binding sites on SLMD and DGT

Table 3 Project Activities

Activities in Integrated Ambient Monitoring Pilot Project at Indian Creek Stations

Actions	Responsible	Timing
Instream Biological Assessments		
benthic invertebrate bioassessment	Ecology	just after end of 1st daphnid deployment
periphyton assessment	Ecology	just after end of 1st daphnid deployment
trout in situ toxicity testing	Nautilus / Ecology	just after benthic and periphyton assessments
trout gene microarray	USGS	on trout after in situ deployment
fish tissue metals	MEL	on trout after in situ deployment
trout metallothionein	Nautilus	on trout after in situ deployment
daphnid in situ toxicity testing	Thurston County / WSU	1st action and repeated 3 times during passive sampler deployment with the last timed to end with passive sampler deployment end
daphnid gene microarray	UC	on daphnids from 1st and last in situ
Laboratory Biological Assessments		
trout exposed to contaminated water	Nautilus	as convenient during project
trout gene microarrays on whole fish and liver	USGS	after trout exposure
trout exposed to estradiol	Nautilus	as convenient during project
trout vitellogenin	Nautilus	after trout exposure
daphnids exposed to downstream samples at 12°and 25°C	Thurston County / WSU	samples taken on 1st and last daphnid in situ exposure
daphnid microarray on daphnids exposed at 12°and 25°C	WSU / UC	after daphnid exposure
Passive Samplers and Analysis		
passive sampler metals - DGT	Brooks Rand / Ecology	for 28 days just after in situ trout deployment
passive sampler metals - SLMD	Brooks Rand / Ecology	for 28 days just after in situ trout deployment
metals analysis - (DGT and SLMD) for **Cd, Cu, Ni, Pb,** and **Zn**	Brooks Rand	after passive sampler retrieved
passive sampler nonpolar - SPMD	EST / Ecology	for 28 days just after in situ trout deployment
extraction SPMD	EST	after passive sampler retrieved
analysis SPMD - **BNAs, PAHs**	MEL	when extract delivered to MEL
analysis SPMD - **pesticides (Cl,OP,N)**	MEL	when extract delivered to MEL
passive sampler polar - POCIS	EST / Ecology	for 28 days just after in situ trout deployment
extraction POCIS	EST	after passive sampler retrieved
analysis POCIS - **herbicides**	MEL	when extract delivered to MEL
analysis POCIS - **pesticides (Cl,OP,N)**	MEL	when extract delivered to MEL
analysis POCIS - **carbamates**	MEL	when extract delivered to MEL
analysis POCIS - **BNAs**	MEL	when extract delivered to MEL
Water Chemistry		
stream grab sample for metals	Ecology	3 times during passive sampler and daphnid deployments
metals analysis - for **Cd, Cu, Ni, Pb,** and **Zn**	Brooks Rand	3 times during passive sampler deployment
analysis BLM parameters - **DOC, pH, Ca, Mg, Na, K, SO_4, Cl, and alkalinity**	MEL	3 times during passive sampler deployment
Biotic Ligand Model (BLM)	Ecology	3 times during passive sampler deployment
MiniSonde physical and chemical measurements	Ecology	on each station visit

Figure 1 Project Timeline

Study Timeline (day 0 = April 12, 2010)

0 DAY 4	8	12	16	20	24	28	32	36	40
benthic invertebrate & periphyton bioassessments									
	bioassessments								
in situ deployments									
		trout 28 to 32 day deployment							genomics, metals, MT
		colonization of rock in baskets							
daphnid	genomics		daphnid		daphnid		daphnid	genomics	
lab trout testing									
		trout 28 to 32 day lab control							genomics
						fry toxic exposure for whole fish vs. liver only comparison			genomics
						fry exposure to estradiol			VTG
lab daphnid testing to stream sample									
test 12°	genomics						test 12°	genomics	
test 25°	genomics						test 25°	genomics	
stream samples for metals and BLM									
			grab	BLM	grab	BLM	grab	BLM	
passive sampler deployment									
		SPMD - 28 days							
		POCIS - 28 days							
		DGT - 28 days							
		SLMD - 28 days							

Table 4 Passive Sampler Analyses

POCIS-only Analytes			DGT/SLMD
Herbicides by GC/MS		**Carbamates**	**Metals**
2,4,6-Trichlorophenol	2,4,5-TP (Silvex)	Methomyl oxime	cadmium
3,5-Dichlorobenzoic Acid	2,4,5-T	Oxamyl oxime	copper
4-Nitrophenol	2,4-DB	Aldicarb Sulfoxide	nickel
Clopyralid	Dinoseb	Aldicarb Sulfone	lead
2,4,5-Trichlorophenol	Bentazon	Oxamyl (Vydate)	zinc
Dicamba I	Ioxynil	Methomyl	
2,3,4,6-Tetrachlorophenol	Picloram	3-Hydroxycarbofuran	
MCPP (Mecoprop)	Dacthal (DCPA)	Imidacloprid	
MCPA	Acifluorfen (Blazer)	Aldicarb	
Dichlorprop	Diclofop-Methyl	Baygon (Propoxur)	
Bromoxynil	Chloramben	Carbofuran	
2,4-D		Carbaryl	
2,3,4,5-Tetrachlorophenol		Methiocarb	
Triclopyr		1-Naphthol	
Pentachlorophenol		Promecarb	

SPMD and POCIS Analytes			
PESTICIDES		**BNAs**	
2,4'-DDD	Fipronil Disulfinyl	1,2,4-Trichlorobenzene	Isophorone
2,4'-DDE	Fipronil Sulfide	1,2-Dichlorobenzene	Naphthalene
2,4'-DDT	Fipronil Sulfone	1,2-Diphenylhydrazine	Nitrobenzene
4,4'-DDD	Fluridone	1,3-Dichlorobenzene	N-Nitrosodimethylamine
4,4'-DDE	Fonofos	1,4-Dichlorobenzene	N-Nitrosodi-n-propylamine
4,4'-DDT	Gamma-BHC (Lindane)	1-Methylnaphthalene	N-Nitrosodiphenylamine
4,4'-Dichlorobenzophenone	Heptachlor	2,4,5-Trichlorophenol	Pentachlorophenol
Acetochlor	Heptachlor Epoxide	2,4,6-Trichlorophenol	Phenanthrene
Alachlor	Hexachlorobenzene	2,4-Dichlorophenol	Phenol
Aldrin	Hexazinone	2,4-Dimethylphenol	Pyrene
Alpha-BHC	Imidan	2,4-Dinitrophenol	Retene
Atrazine	Kelthane	2,4-Dinitrotoluene	Triclosan
Azinphos-ethyl	lambda-Cyhalothrin	2,6-Dinitrotoluene	Triethyl citrate
Azinphos-methyl (Guthion)	Linuron	2-Chloronaphthalene	
Benefin	Malathion	2-Chlorophenol	
Benthiocarb	Metalaxyl	2-Methylnaphthalene	
Beta-BHC	Methidathion	2-Methylphenol	
beta-Cypermethrin	Methoxychlor	2-Nitroaniline	
Bifenthrin	Methyl Chlorpyrifos	2-Nitrophenol	
Bromacil	Methyl Paraoxon	3,3'-Dichlorobenzidine	
Butachlor	Methyl Parathion	3B-Coprostanol	
Butylate	Metolachlor	3-Nitroaniline	
Captan	Metribuzin	4,6-Dinitro-2-Methylphenol	
Carboxin	Mevinphos	4-Bromophenyl phenyl ether	
Chlorothalonil (Daconil)	MGK264	4-Chloro-3-Methylphenol	
Chlorpropham	Mirex	4-Chloroaniline	
Chlorpyrifos O.A.	Monocrotophos	4-Chlorophenyl-Phenylether	
Chlorpyriphos	Naled	4-Methylphenol	
cis-Chlordane	Napropamide	4-Nitroaniline	

Cis-Nonachlor	Norflurazon	4-Nitrophenol
cis-Permethrin	Omethoate	4-nonylphenol
Coumaphos	Oryzalin	Acenaphthene
Cyanazine	Oxychlordane	Acenaphthylene
Cycloate	Oxyfluorfen	Anthracene
Dacthal (DCPA)	Parathion	Benzo(a)anthracene
Delta-BHC	Pebulate	Benzo(a)pyrene
Deltamethrin	Pendimethalin	Benzo(b)fluoranthene
Di-allate (Avadex)	Phenothrin	Benzo(ghi)perylene
Diazinon	Phorate	Benzo(k)fluoranthene
Diazinon O Analog	Phorate O.A.	Benzoic Acid
Dichlobenil	Phosmet O.A.	Benzyl Alcohol
Dichlorvos (DDVP)	Prometon (Pramitol 5p)	Bis(2-chloro-1-methylethyl) ether
Dieldrin	Prometryn	Bis(2-Chloroethoxy)Methane
Dimethoate	Pronamide (Kerb)	Bis(2-Chloroethyl)Ether
Diphenamid	Propachlor (Ramrod)	Bis(2-Ethylhexyl) Phthalate
Disulfoton (Di-Syston)	Propargite	Bisphenol A
Disulfoton Sulfone	Propazine	Butyl benzyl phthalate
Disulfoton Sulfoxide	Resmethrin	Caffeine
Diuron	Simazine	Carbazole
Endosulfan I	Simetryn	Cholesterol
Endosulfan II	Sulfotepp	Chrysene
Endosulfan Sulfate	Tebuthiuron	Dibenzo(a,h)anthracene
Endrin	Terbacil	Dibenzofuran
Endrin Aldehyde	Tetrachlorvinphos	Diethyl phthalate
Endrin Ketone	Tokuthion	Dimethyl phthalate
EPN	Tralomethrin	Di-N-Butylphthalate
Eptam	trans-Chlordane	Di-N-Octyl Phthalate
Ethalfluralin (Sonalan)	Trans-Nonachlor	Ethanol, 2-Chloro-, Phosphate (3:1)
Ethion	trans-Permethrin	Fluoranthene
Ethoprop	Treflan (Trifluralin)	Fluorene
Fenamiphos	Triadimefon	Hexachlorobenzene
Fenamiphos Sulfone	Triallate	Hexachlorobutadiene
Fenarimol	Trichloronate	Hexachlorocyclopentadiene
Fenvalerate (2 isomers)	Tricyclazole	Hexachloroethane
Fipronil		Indeno(1,2,3-cd)pyrene