Program and Abstracts

Pacific Northwest Chapter

Society of Environmental Toxicology and Chemistry

(PNW-SETAC)

21st Annual Meeting

Downtown Vancouver, British Columbia, Canada.

April 26-28, 2012

Sandman Hotel Vancouver City Centre
180 W. Georgia Street
Vancouver, B.C., Canada

Cover photo accessed on February 1, 2012 at:
http://carolguess.blogspot.com/2010/10/cross-border-reading-in-vancouver-bc.html

PNW-SETAC ANNUAL MEETING

April 26 to 28, 2012

Meeting Program

PNW-SETAC
Chapter Meeting Agenda

Sandman Hotel Ballroom

Thursday, April 26, 2012

12:00 PM - 7:30 PM	**Conference/Registration Check-in** (Ballroom Entrance)
1:00 PM - 5:30 PM	**Short Course 1**: *Application of Data & Models to Develop Ecologically Relevant Sediment Quality Criteria* by **Dr. Frank Gobas**, SFU
	Short Course 2: *Environmental Chemistry for Site Reclamation and Remediation Made Easy* by **George (Bud) Ivey**, Ivey International Inc.
5:30 PM - 8:00 PM	Welcome Reception with Refreshments
7:00 PM - 8:00 PM	PNW-SETAC Board Meeting

Friday, April 27, 2012

7:30AM – 5:30 PM	**Conference/Registration Check-in** (Ballroom Entrance)
7:30AM - 8:00 AM	Poster setup
8:15 AM - 9:00 AM	Welcome address, Chapter President Vicki Marlatt Report from SETAC NA, SETAC NA Board member John Elliot
9:00 AM - 6:00 PM	Poster viewing
9:00 AM – 12:00 PM	**Special Session**: *Wildlife Ecotoxicology*, 20 min break for refreshments, poster viewing
12:00 PM-1:30 PM	**Lunch, on your own**
12:40 PM - 1:25 PM	Chapter Business Meeting, **All Welcome to Attend!!**
1:30 PM - 5:00 PM	Special Session cont., with other platform sessions afterwards and a 30 min break for refreshments and poster viewing
5:00 PM - 6:00 PM	Poster Social
6:00 PM - 8:30 PM	Buffet Dinner

Saturday, April 28, 2012

7:30 AM	**Conference/Registration Check-in** (Ballroom Entrance)
8:30 AM - 12:30 PM	Platform sessions with 20 min break for refreshments and poster viewing
12:35 PM	Student Award Presentations
1:00 PM	Adjourn

PNW-SETAC
Meeting Sponsors

Special thanks to all our meeting sponsors!!

Program Printing

Anchor QEA, LLC

Student Travel Funds, Student Awards

Azimuth Consulting Group
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Thursday Evening Welcome Reception

Nautilus Environmental
Maul Foster & Alongi, Inc.

Friday Morning Breakfast and Refreshments

Nautilus Environmental
CH2M Hill

Friday Afternoon Refreshments

ALS Environmental/ALS-Columbia
Maxxam Analytics

Saturday Morning Breakfast and Refreshments

Maul Foster & Alongi, Inc.
Exponent

PNW-SETAC
Sustaining Corporate Members

Please join us in welcoming our first (of hopefully many) Sustaining Members!!

Maul Foster & Alongi, Inc. (MFA) (http://www.maulfoster.com) is an integrated multidisciplinary professional services organization consulting in the areas of environmental science, engineering, planning, landscape architecture, and GIS. We are approaching our 16th year of providing services to a diverse client base that includes private business and industry, ports, municipalities, and Native American tribes.

MFA is a locally owned Pacific Northwest consulting firm established in Vancouver, Washington by Jim Maul, Tom Foster, and Neil Alongi. Since our inception in 1996, MFA has grown from four employees to more than 70 employees today with offices in Portland, Oregon and in Vancouver, Seattle, and Bellingham, Washington. We are also in the process of opening an office in Kellogg, Idaho. We are committed to a better planned, well designed, and greener future.

Nautilus Environmental (http://www.nautilusenvironmental.com) is an environmental services company focused on providing high quality scientific data in the field of environmental toxicology.

Our team of environmental scientists has expertise in the fields of environmental toxicology, chemistry, biology, and ecology, which ensures that studies conducted by our company are designed, implemented and interpreted in a manner that maximizes the value of the data. Nautilus has developed a renowned program that applies both standardized and innovative methodologies to address a range of toxicological concerns.

At the core of the business are our accredited environmental toxicology laboratories in Vancouver, B.C. and San Diego, CA, each offering a wide range of toxicity testing services. Please explore our site or contact us to learn more about how we can assist you.

To learn more about the benefits of becoming a Sustaining Corporate Member, please visit our web site at http://www.pnw-setac.org/Sustaining.htm

PNW-SETAC
Acknowledgements

Thanks to all of the following who volunteered their time to make this meeting possible:

Conference Organization:	Vicki Marlatt, University of the Fraser Valley Curtis Eickhoff, Maxxam Analytics Jerome Laroulandie, Maxxam Analytics
Short Course 1:	Frank Gobas, Simon Fraser University
Short Course 2:	George (Bud) Ivey, Ivey International Inc.
Special Session:	John Elliott, Environment Canada Christine Bishop, Environment Canada
Reservations/Food:	Vicki Marlatt, University of the Fraser Valley Teresa Michelsen, Avocet Consulting
On-Site Coordinators:	Vicki Marlatt, University of the Fraser Valley Ryan Loveridge, CH2M Hill
Abstract Review:	Lesley Shelley, Simon Fraser University Patrick Moran, USGS Ryan Loveridge, CH2M Hill
Meeting Program:	April Markiewicz, Western Washington University
Meeting Registration:	Ryan Loveridge, CH2M Hill
Volunteer Coordinator:	Lesley Shelley, Simon Fraser University
Fundraising:	PNW-SETAC Board of Directors
Session Chairs:	John Elliott, Environment Canada Christine Bishop, Environment Canada Mark Jankowski, Lewis-Clark State College Maggie Dutch, WA Department of Ecology
Student Awards Coordinator:	Ruth Sofield, Western Washington University Heather Henson-Ramsey, Lewis-Clark State College

Student Award Judges:	Juan Jose Alava, Simon Fraser University Alan Bergmann, Oregon State University Brian Church, Windward Environmental Jean-Pierre Desforges, University of Victoria Aaron Edgington, Oregon State University Heather Henson-Ramsey, Lewis-Clark State College Peter Kickham, B.C. Ministry of Environment Lucy Lopez Francini Martins, Keystone Environmental Ltd. Jenifer McIntyre, Washington State University Patrick Moran, US Geological Survey Madi Novak, Maul Foster & Alongi Heather Osachoff, Simon Fraser University Lesley Shelley, Simon Fraser University Burt Shephard, US EPA Region 10 Siobhan Sloan-Evans, Pacific Northwest National Laboratory Fran Solomon, Environmental Teaching International Jeff Wirtz, Compliance Services International
Student Travel Review:	Ruth Sofield, Western Washington University Heather Henson-Ramsey, Lewis-Clark State College
Media Equipment:	April Markiewicz, Western Washington University Vicki Marlatt, University of the Fraser Valley

Sandman Hotel Vancouver City Centre, Vancouver, B.C., Canada Location and Vicinity Map

Accessed February 6, 2012 at http://www.hotels.com/ho112511/sandman-hotel-vancouver-city-centre-vancouver-canada/#maps

Next Year's Meeting!

NORTHERN QUEST RESORT & CASINO

22nd Annual PNW-SETAC Meeting

Thursday April 18-20, 2013

Northern Quest Resort and Casino

Airway Heights, WA (~20 miles east of Spokane)

Please mark your calendars for our 2013 PNW-SETAC Annual Meeting to be held at the Northern Quest Resort and Casino (http://www.northernquest.com) just 20 miles east of Spokane. This jewel in eastern Washington is rated "four diamonds" and offers spacious meeting rooms, fine dining, live entertainment, a world class spa, and (of course) a casino to test your skills in courting Lady Luck.

Plans for our 2014 annual meeting are also underway and we are currently obtaining bids from venues in the Tacoma, WA area. More details will be forthcoming in the coming year.

Pacific Northwest Chapter

Society of Environmental Toxicology and Chemistry

(PNW-SETAC)

21st Annual Meeting

Pre-Meeting Events

PNW-SETAC
Thursday Short Course I

1:00 PM, Thursday, April 26, 2012

Sandman Hotel Ballroom

Application of Data & Models to Develop Ecologically Relevant Sediment Quality Criteria

Instructor: Frank Gobas, Simon Fraser University

Rationale:
Sediment quality criteria (SQC) for contaminants are widely used by government agencies and consultants for a range of purposes, including conducting risk assessments, setting goals for remediation and setting permits for ocean disposal of contaminated sediments. In most cases, the sediment quality guidelines are based on toxicity information for benthic invertebrates and hence aim to protect benthic invertebrate populations. The sediment quality criteria are in most cases not suitable to protect other organisms such as fish, crabs, marine mammals, birds and human populations who may consume various organisms through fishing, crabbing and other forms of hunting.

As a result there is a need to develop sediment quality criteria for contaminants that are more ecologically relevant, i.e. protect a larger range of species of wildlife and also human populations who consume wildlife.

Objective:
The objective of this short course is to discuss the methods that can be used to develop sediment quality criteria for contaminants that have greater ecological and human health relevance. Specifically, the course will address the application of contaminant concentration data and food and food web bioaccumulation models to determine sediment quality criteria for contaminants.

Course Outline:

Introduction (20 min.)
Part 1: Theory and practice of current sediment quality criteria (SQC) (20 min.)
Part 2: Benefits and limitations of current SQC (20 min)
Part 3: Methods for developing SQC for multiple ecological and human receptors (40 min.)
Part 4: Application of empirical concentration data for SQC development (45 min.)
Part 5: Application of Food-Web Bioaccumulation Models for SQC development (45 min.)
Part 6: Summary (15 min.)
Part 7: Open discussion (40 min.)

Contact Info: Frank Gobas, Simon Fraser University
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PNW-SETAC
Thursday Short Course II

1:00 PM, Thursday, April 26, 2012

Sandman Hotel Ballroom

Environmental Chemistry for Site Reclamation and Remediation Made Easy:

A New Perspective on Water, Contaminant Solubility, Sorption, and New Innovative Tools for your Remediation and Reclamation Toolbox

Instructor: George (Bud) Ivey, President, Ivey International Inc.

Overview:

This short course will introduce attendees to a new and easy to use set of principles regarding soil and groundwater contamination and hydrogeology, with a perspective not taught in High School or Universities. This is achieved through a visually driven and interactive hands-on presentation in which they learn: water is not H2O, what really affects contaminant solubility and the important role of contaminant sorption (i.e., absorption and adsorption) in soil and groundwater hydrogeology, as this relates to characterization and remediation. Attendees with limited chemistry, biochemistry, microbiology or hydrogeology experience will lean a new set of principles to accurately predict the behaviour of LNAPL and DNAPL contaminants in soil and groundwater include their water solubility, sorption potential, how to improve their physical and or chemical availability for in-situ or ex-situ remediation at most sites.

The presentation will challenge conventional understandings (Models) of what water and organic contaminants are, and provide three simple tools to predict their behaviour in water, with each other, and with soils and bedrock. In doing so, this simplifies many contaminant hydrogeology principles through better understanding why some contaminants dissolve in water while others do not, and why some absorb or adsorb to soil while others do not, why some are less 'available' for remediation, while others are not. Attendees will leave the workshop with an applied ability to predict contaminant behaviour as it affects site investigation and remediation action plans.

Over the course of the presentation attendees also learn the #1 factor limiting the effectiveness of all soil and ground water bioremediation technologies. They will also learn about the use of a new class of surfactants in remediation, surfactant types, their selection, application pros and cons, and how they can serve as an innovative tool in their soil and groundwater bioremediation toolbox.

Contact Info: George (Bud) Ivey, Ivey International Inc.
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W: www.iveyinternational.com

Pacific Northwest Chapter

Society of Environmental Toxicology and Chemistry

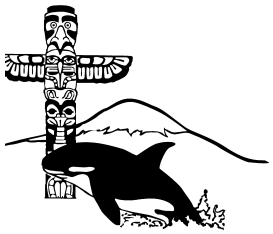
(PNW-SETAC)

21st Annual Meeting

Schedule of Platform and Poster Presentations

PNW-SETAC

Friday Morning Special Session



Wildlife Ecotoxicology

Session Chairs: **John Elliott** and **Christine Bishop**, Environmental Canada

Wildlife Ecotoxicology: Snakes, Frogs and Birds

9:00	Christine Bishop	Impact assessment of Gopher Getter, a rodenticide containing strychnine, on Great Basic gophersnakes (*Pituophis catenifer deserticola*) in British Columbia's Okanagan valley
9:20	Brittany Wilmot	An *in situ* study of current use pesticides on the growth, development and gene expression of *Rana luteiventris* in the Okanagan valley, British Columbia
9:40	Mark Jankowski	Studies of how exposure route might alter the effects of pyrethroids on avian immunity to West Nile Virus
10:00	Sofi Hindmarch	Investigating the potential risk of secondary rodenticide poisoning to urban owls inhabiting and foraging in urban landscapes of the Lower Mainland, British Columbia
10:20	**Break/Poster Viewing**	

Wildlife Ecotoxicology: Birds

10:40	Toby St. Clair	Metal exposure to dunlins (*Calidris alpina*) in the Fraser River delta, British Columbia
11:00	Mikaela Davis	Dietary trends of the glaucous-winged gull on the Canadian west coast: context for interpretation of contaminant-monitoring data
11:20	Margaret Eng	Early exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) affects neuroanatomy and behavior of zebra finches
11:40	Heidi Scherr	*In vitro* screening, and use of an animal model the zebra finch, to identify possible endocrine disrupting effects of 1,2-dibromo-4-(1,2-dibromomethyl) cyclohexane
12:00 to 1:30 PM		**Lunch**
12:40 to 1:30 PM		**PNW-SETAC Business Meeting**

PNW-SETAC
Friday Afternoon Sessions

Friday April 27, 2012

Session Chairs: **John Elliott** and **Christine Bishop**, Environmental Canada

Wildlife Ecotoxicology Continued: Marine Mammals

1:30	Peter Ross	Persistent organic pollutants (POPs) in the NE Pacific Ocean: Lessons learned from endangered killer whales
1:50	Heloise Frouin	Contaminants and immunotoxicity in pinnepeds and cetaceans
2:10	Jean-Pierre Desforges	Transfer of PBCs and PBDEs from mother to fetus in beluga whales (*Delphinapterus leucas*) from the western Canadian arctic
2:30	Cait Nelson	An assessment of exposure and effects of persistent contaminants in river otters (*Lontra canadensis*) in Victoria harbor, British Columbia

2:50 Break/Poster Viewing

Session Chair: **Mark Jankowski**, Lewis-Clark State College

Developments in Toxicology

3:20	Pola Wojnarowicz	Evaluating the Efficacy of Three Parallel Secondary Municipal Wastewater Treatment Systems with Transcriptomic Endpoints of a Bullfrog C-Fin Assay
3:40	Paula Duarte-Guterman	Regulation of Estrogen- and Androgen-Related Genes in Developing Frogs: Implications for Endocrine Disruption
4:00	Claudio Erratico	CYPB6 is the only CYP Enzyme Responsible for the Oxidative Metabolism of BDE-99 by Human Liver Microsomes
4:20	Ruth Sofield	Interactions of Humic and Fulvic Acids with Silver Nanoparticles and the Resultant Toxicity to *Chlamydomonas reinhardtii*
4:40	Bonnie Lo	The Effect of Sulphate on the Aquatic and Dietary Uptake of Selenium in the Water Flea, *Daphnia magna*

5:00 Poster Social

6:00 **Dinner**

PNW-SETAC

Saturday Morning Sessions

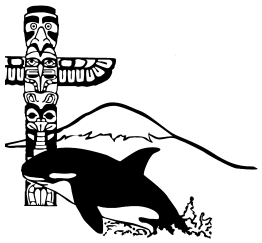
Saturday April 28, 2012

Session Chair: **Maggie Dutch**, Washington State Department of Ecology

Population models, Risk Assessment and Sediment Toxicity

8:30	John Stark	Advances in the use of Population Models for Ecological Risk Assessment of Pesticides
8:50	Les Williams	Using Spatially Explicit Data and Modeling to Inform Ecological Risk Assessment for a Proposed Pit Mine Expansion
9:10	Eleanor Hines	Regional Risk Assessment of The Puyallup River Watershed And Low Impact Development (LID) to Meet Management Goals
9:30	Bruce Hope	An Exploration of Exposure Scenarios for Anadromous Fish
9:50	Matt Luxon	Development of a Spatially Explicit Population Model for PCB Exposed Mink
10:10	Jenifer McIntyre	Biological Effectiveness of Green Stormwater Infrastructure: Assessing Sublethal Impacts of Urban Stormwater Runoff to Developing Fish and Invertebrates
10:30	**Break**	
10:50	Maggie Dutch	Development of a New Sediment Chemistry Status and Trends Index for Environmental Management in Puget Sound, WA
11:10	Frank Gobas	Towards Ecosystem Based Sediment Quality Criteria
11:30	Jonathan Benskin	Occurrence and Biodegradation of a Novel PFOS-Precursor, The Perfluorooctane Sulfonamido Ethanol-Based Phosphate Diester (SAM-PAP) in Marine Sediments
11:50	Wayne Landis	Introduction to the Calculation of Risks due to Mercury and other Stressors to Multiple Endpoints for the South River and Upper Shenandoah River, Virginia and the Projected Outcomes of Different Management Scenarios
12:10	Lucinda Tear	Ubiquitous, Flying Outliers – Skewness and its Ramifications for BSAFs, Background Threshold Values, and Site Conceptual Models
12:30	**Student presentation awards**	
1:00	**Wrap up/Adjourn**	

PNW-SETAC
Poster Presentations



Presenter(s)	Presentation
Kimberley Ayre	The Use of Bayesian Network Models to Evaluate Ecological Risk from Hg Contamination in the South River, VA
Kimberley Ayre	An Assessment of Risk to Water Quality and Ecological Services for the South River, VA
Krysta Banack	Evaluation of Toxicity Modifying Factors for Fluoride Using *Hyalella Azteca* and Rainbow Trout
Aimée Brisebois	Exploring the Relationship Between the BCF BAF and the TMF
Jason Brogan	Persistent Organic Pollutant Concentrations in Urban Raptors of British Columbia
Keith Brown	Determining the Additive, Synergistic or Antagonistic Effects of Chemical Mixtures of Carbaryl And Malathion on *Daphnia magna*
Rachelle Combs Craig Murdock	Toxicity of Silver Nanoparticles with Three Capping Agents (PVP, Citrate, and CO_3): An Evaluation of Ionic Versus Total Silver
Susannah Edwards	Using a Bayesian Network Approach to Evaluate the Effectiveness of Low Impact Development Techniques in Managing Risk in the Puyallup River Watershed
Tyler Ellis	Does EPA Method 7471 Accurately Measure Mercury Concentrations in Fish?
Jacqueline Ford Therese Crowell	Influence of Temperature on Heavy Metal Toxicity to *Lemna turionifera*
Timothy Gray	The Activity of P-Glycoprotein is Not Regulated by Oxidative Stress in Isolated Rainbow Trout (*Oncorhynchus mykiss*) Hepatocytes
Jessica Huybregts	Rapid Risk Assessment: Exploratory Analyses Using An Excel-Based Eco-Risk Tool

PNW-SETAC
Poster Presentations

Presenter(s)	Presentation
Donald Lao	The Acute Toxicity of Antihistamines to Snails, *Lymnaea stagnalis*
Justin Lo	A Model for Assessing the Bioaccumulation Potential of Metabolizable Hydrophobic Substances in Rainbow Trout (*Oncorhynchus mykiss*)
Alexander MacLeod	Investigation into Toxicity of Chemical Mixtures Involving Organophosphate Pesticides: Malathion And Diazinon Toxicity to *Daphnia magna*
Jenifer McIntyre	Effects of Urban Stormwater Runoff on Macroinvertebrate Communities and Implications for Endangered Salmonids
Jenifer McIntyre	Estimating Impacts on Coho Salmon Populations from Land Use-Related Spawner Mortality in Urban Streams
Sarah Moorman Jonnel Deacon Anthony Gibson	Determination of the Acute Toxicity of Benzene to *Eisenia fetida* in Artificial Soil Over 7 Days
Leslie Saunders	The Effect of Pre-Exposure On *In Vitro* Biotransformation Rates of Hydrophobic Chemicals in Rainbow Trout (*Oncorhynchus myskiss*)
Andrew Schroeder	Thyroid Gland Histopathology of a Native North American Species, *Pseudacris regilla,* in the Amphibian Metamorphosis Assay
Jen Stiles Geoffrey Lowery Charles Hixon	The Effect of Atrazine and Roundup® on *Daphnia magna*
Jonah Stinson	Applications of Geographic Information Systems to Ecological Risk Assessment and Modeling in the South River and Upper Shenandoah River, Virginia
Frieda Taub	O_2, Co_2, and Pressure in Aquatic Closed Ecological Systems

PNW-SETAC
Poster Presentations

Presenter(s)	Presentation
Viktoria Winter	A Three-Generational Study of In Ovo Exposure to PBDE-99 in Zebra Finch (*Taeniopygia guttata*)
David Wood Rachelle Combs	Contamination and Toxicity of Snowpack Collected from Snowmobile Recreation Areas

Pacific Northwest Chapter

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Special Session Platform and Poster Abstracts

IMPACT ASSESSMENT OF GOPHER GETTER, A RODENTICIDE CONTAINING STRYCHNINE, ON GREAT BASIN GOPHERSNAKES (*PITUOPHIS CATENIFER DESERTICOLA*) IN BRITISH COLUMBIA'S OKANAGAN VALLEY

Williams, K. [1];Bishop, C.A* [2]; and Elliott, J.E.[2], [1] Van Hees Environmental Consultants, Twisp, WA, [2]Environment Canada, Science and Technology Branch, 5421 Robertson Road, Delta, British Columbia, Canada

Vineyards and orchards currently represent a significant proportion of the land cover in the Okanagan valley BC. Rodents, such as pocket gophers (*Thomomys talpoides*), eat young roots and chew bark which can girdle the tree or vine. Strychnine, the active ingredient in the product Gopher Getter, is put down holes and tunnels to kill the pocket gophers and bring their populations back under control. Great Basin gophersnakes (*Pituophis catenifer deserticola)* are known to eat pocket gophers across their range. We created a model to estimate the exposure of gophersnakes to strychnine, incorporating for example, feeding frequency, prey composition and amount, body mass, and lethal doses of strychnine for pocket gophers and gophersnakes. We evaluated dose at 2 levels, indicating the lowest and highest probable numbers of gophersnakes impacted by strychnine in the Okanagan Valley. We obtained various GIS layers to visualize the extent of the various factors involved, including TEM (terrestrial ecosystem mapping) maps of the south Okanagan and partial TEM maps of the central and north Okanagan, Ministry of Agriculture land use coverage of orchards and vineyards in the Okanagan south of the Peachland area, and a TEM gophersnake habitat suitability model. Data was obtained from the pesticide division of Ministry of environment in Penticton, BC, from vendors local to the south Okanagan. We obtained records for two years from 2005 – 2006 and averaged yearly data to obtain our yearly volume sold (1712 kg). We estimated that between 72 and 3392 gophersnakes could be poisoned per year by Strychnine exposure through consumption of poisoned pocket gopher prey.

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AN *IN SITU* STUDY OF CURRENT USE PESTICIDES ON THE GROWTH, DEVELOPMENT AND GENE EXPRESSION OF *RANA LUTEIVENTRIS* IN THE OKANAGAN VALLEY, BRITISH COLUMBIA.

Wilmot, B. [1],* Bishop, C.A. [2], Nicholson, R.A. [1], Skirrow, R. [2], VanAggelen, G. [2], and Kennedy, C.J. [1].
[1]Simon Fraser University, Burnaby, BC. [2]Environment Canada,, Vancouver, BC.

Amphibians are sentinel species of the aquatic environment and can be used as indicators for risk assessment and management. We studied the use of current use pesticides on amphibian populations in the Okanagan valley through the parallel use of *in situ* field assays and genomic laboratory studies. Our study sites where located in orchard ponds which were sites of known pesticide contamination and that were previously examined for deleterious effects in eggs and tadpoles. The predominant chemical contaminants were identified in source water using ultra-low detection methods. Gene expression of the Columbia spotted frog (*Rana luteiventris)* in the brain and liver was assessed using Q-PCR techniques in frogs at metamorphosis. Some genes of interest include, thyroid hormone receptor-beta (THRB), estrogen receptor (ERA), androgen receptor (AR), and superoxide dismutase (SOD) play vital roles in the growth and development of amphibians. Significant reductions were seen on hatching success, survivorship and size at in-situ orchard pond sites. These observed deleterious effects significantly correlated with gene expression data. This field and laboratory data will allow a better understanding of the cumulative nature of chemical, biological, physical and emerging pollutants on the aquatic environment.

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STUDIES OF HOW EXPOSURE ROUTE MIGHT ALTER THE EFFECTS OF PYRETHROIDS ON AVIAN IMMUNITY TO WEST NILE VIRUS

Jankowski, M.D.*[,1], Hofmeister, E.K.[2], Moore, M.E.[1], and Brownawell, B.[3], [1]Los Alamos National Laboratory, Los Alamos, NM, [2]U.S. Geological Survey, National Wildlife Health Center, Madison, WI, [3]SUNY-Stonybrook School of Marine and Atmospheric Sciences, Stony Brook, NY

Synthetically derived pyrethrins, pyrethroids, are commonly used insecticides in agricultural and public health settings. When local mosquito populations are infected with an arbovirus, such as West Nile virus (WNV), mosquito control agencies may deploy synergized formulations of pyrethroids such as resmethrin and permethrin in order to reduce the risk of human infection. Local avifauna are inevitably exposed. Our group is therefore investigating how water and airborne pyrethroid exposure differentially affect the avian immune response to WNV. Our central question concerns whether the exposure to pyrethroids can enhance avian host competence – viremia – by way of immune suppression, and thus amplify local WNV transmission cycles. We previously determined that in WNV infected chickens, three days of waterborne exposure to synergized resmethrin reduced the humoral immune response, elevated oral viral shedding, and extended viremia by one day. Our current work involves the development of a particle size selective aerosolizer and chamber for the exposure of passerines to mists of synergized permethrin. We have achieved the ability to produce aerosol plumes with aerodynamic diameters of 1, 10, 20 µm using a cyclone. Subsequently, we are working towards achieving environmentally relevant chamber insecticide concentrations ranging from 0.1 to 218 ppb permethrin. Once completed, this information will help mosquito control professionals decide how specific spraying protocols can be used to limit the negative effects of insecticides on local avifauna.

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THE POTENTIAL RISK OF SECONDARY RODENTICIDE POISONING TO URBAN OWLS INHABITING AND FORAGING IN URBAN LANDSCAPES OF THE LOWER MAINLAND, BRITISH COLUMBIA

Hindmarch, S.R.* and Elliott, J.E., Environment Canada, Delta, B.C., Canada.

Anticoagulant rodenticides are widely used to control pest rodents, but poisoning of non-target wildlife has been linked to these practices, including secondary poisoning of birds of prey, particularly owls. In this study, we investigate whether Barred owls (*Strix varia*), Great-horned owls (*Bubo virginianus*) and/or Barn owls (*Tyto alba*) inhabiting and foraging in predominantly urban landscapes of the Lower Mainland, British Columbia are at risk of consuming rodenticide-laden prey, such as rats and house mice. By conducting a pellet study, we found that urban Barred owls had the largest proportion of rats in their diet, with some individuals' diet consisting primarily of rats. Urban Great-horned owl pellets were also comprised mainly of rats, but there was a clear shift towards alternative prey base when urbanization within home ranges decreased. Field voles (*Microtus townsendi*) were the main prey item for Barn owls, regardless of the amount of urbanization within their home range. For all three species, consumption of rats and house mice appears to coincide with increased urbanization within home ranges. The shift in the diet of owls living in urbanized areas may potentially lead to an increased risk of secondary rodenticide poisoning. Radio telemetry was deployed to further investigate which landscape features urban Barn owls select as foraging habitat and whether they forage in proximity to buildings where rodenticide is applied. Urban Barn owls were found to predominantly forage in grass strips along highway interchanges and verges, and untended grass patches within the city. The majority of foraging was done within 100 m of commercial buildings where rodenticide had been applied. These findings will be discussed in conjunction with previous research done on rodenticide residues found in the livers of deceased owls and the current and historic sales of rodenticides in the Lower Mainland, BC.

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METAL EXPOSURE TO DUNLINS (CALIDRIS ALPINA) IN THE FRASER RIVER DELTA, BRITISH COLUMBIA

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Estuaries receive deposition of suspended sediments and organic matter from aquatic systems. Heavy metals bound to such deposits accumulate in estuarine sediments. For coastal biota, risk of exposure to toxic quantities of metals is further elevated in coastal regions with dense human populations. Dunlins (*Calidris alpina*) are migratory sandpipers that feed variably in intertidal and agricultural areas on biofilm, insects, worms, gastropods, and other marine invertebrates. We analyzed cadmium, copper, and zinc levels in Dunlin gizzard contents from individuals captured in the Fraser River Delta, adjacent to Vancouver, British Columbia, to assess the level of exposure and investigate the contribution of metals from different prey and habitat types. Daily metal exposure was calculated based on energetic needs (120-130 Kj/day), and the caloric concentrations of food items relative to metal concentrations. Cadmium exposure was greater for Dunlin foraging in intertidal habitat as compared to those feeding in agricultural habitat. While ingested biofilm and associated sediments had significantly higher cadmium concentrations than all other food types (7.4 µg/g ± 1.9(SD)), *Batilleria* mud snails had a slightly higher amount of cadmium relative to their energetic contribution to Dunlin diet (0.74 µg/Kj ± 0.15(SD)). Daily metal exposure was found to be lower than LOAELs for all metals, but higher than NOAELs of cadmium for an all mud snail diet in Boundary Bay, BC and higher than zinc NOAELs for a biofilm diet and terrestrial diet at the Vancouver International Airport.

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DIETARY TRENDS OF THE GLAUCOUS-WINGED GULL ON THE CANADIAN WEST COAST: CONTEXT FOR INTERPRETATION OF CONTAMINANT-MONITORING DATA

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Monitoring programs to track contaminant levels in the environment are critical to understanding variation in pollutant concentrations, exposure routes, and spatial and temporal contaminant trends. Persistent organic pollutants (POPs) accumulate in the tissues of high trophic level predators, such as seabirds. Gulls (*Larus* spp.) have been used for over 35 years to monitor POPs in the Great Lakes. Recently, an ecosystem monitoring program utilizing the Glaucous-winged Gull (*Larus glaucescens*) has been launched to track legacy and emerging contaminants on the west coast of Canada. However, the utility of the Glaucous-winged Gull as a marine monitoring species hinges on its consumption of a marine-based diet, and there is a lack of recent and reliable data on diet for this species. Using conventional diet analysis and stable isotope methods, we are studying diet at 3 breeding colonies, and comparing findings with historical data. Results suggest that current adult diet varies from historical records at colonies closer to urban centers. Compared to studies in 1982, adult diets from 2010 included a lower prevalence of anthropogenic diet sources and elevated marine sources. In contrast, adult diet on the west coast of Vancouver Island is consistent with historical data, remaining marine-based. Chick diet varied little between 1982 and present years, with fish dominating at all colonies, though anthropogenic items were still observed at colonies near urban areas. Dietary findings will be presented along with current toxicological results, and the feasibility of using the Glaucous-winged Gulls as a marine biomonitoring tool will be discussed.

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EARLY EXPOSURE TO 2,2′,4,4′,5-PENTABROMODIPHENYL ETHER (BDE-99) AFFECTS NEUROANATOMY AND BEHAVIOR OF ZEBRA FINCHES

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2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) is a brominated flame retardant congener that has pervaded global food chains, being reported in avian egg and tissue samples throughout the world. Its effects on birds are not well known, but there is evidence in exposed mammals that it directly mediates and causes neurotoxicity, alters thyroid hormone homeostasis, and lowers sex steroid hormone concentrations. In birds, those processes could disrupt the song-control system and male mating behavior. In this study, the effects of nestling exposure to environmentally relevant levels of BDE-99 were assessed in a model songbird species, the zebra finch (*Taeniopygia guttata*). A tissue residue study in which zebra finch nestlings were orally exposed to 0, 2.5, 15.8, or 50.7 ng BDE-99/g bw/day over the 21-day nesting period validated dosing methods and confirmed dose levels were environmentally relevant (332.7 ± 141.0 to 4450.2 ± 1396.2 ng/g plasma lipid). A full scale study exposing nestlings to 0, 2.5, 15.8, 50.7 or 173.8 ng BDE-99/g bw/day was carried out to investigate long-term effects of BDE-99 on the adult song-control nuclei volumes, song quality, and male mating behavior. Early exposure to BDE-99 had significant effects on female song-control nuclei, male mating behavior, and the response of unexposed experienced females to exposed males. There was no effect on male song-control nuclei or song quality. The results demonstrate that early exposure to environmentally relevant levels of BDE-99 affects the neuroanatomy and behavior of zebra finches.

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IN VITRO SCREENING AND USE OF AN ANIMAL MODEL, THE ZEBRA FINCH, TO IDENTIFY POSSIBLE ENDOCRINE DISRUPTING EFFECTS OF 1,2-DIBROMO-4-(1,2-DIBROMOETHYL) CYCLOHEXANE

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Many brominated flame retardants (BFRs) can be classified as endocrine disrupting compounds. Various health problems in both humans and animals have been attributed to BFRs. One BFR, TBECH (1,2- dibromo-4-(1, 2-dibromoethyl) cyclohexane), has recently been detected in the environment and biota, but very little is known of its environmental impacts. To identify whether TBECH has any endocrine disrupting capabilities, it was run through an *in vitro* screening process that included real-time PCR techniques in two different cell lines (human endometrial carcinoma cell line ECC-1, and human prostate cancer cell line LNCaPs), and use of the CALUX assay in the human breast cancer cell line T-47Luc4ARE (Androgen Response Element CALUX). We were interested in whether TBECH was able to cause expression in human androgen, estrogen, progesterone, and aryl hydrocarbon receptors. All cells were treated with environmentally comparable levels of TBECH alone and in combination with the respective steroid hormones to determine if the compound acts as an agonist, antagonist, or amplifies the effects of the hormones. RNA extraction and real-time PCR techniques were used to measure any gene expression that occurred. For the T-47D CALUX cell line, androgen expression was measured by the concentration of proteins produced from activation. Initial results indicate that TBECH acts as an antagonist on the androgen receptor in both the LNCaP and T-47Luc4ARE cell-lines, contrary to other findings. To see how these results might affect growth and development in an animal model, TBECH was then tested in zebra finches. Finch embryos were exposed to TBECH via in ovo dosing procedures, and their growth and development was measured to fledging. No significant effects on growth, development, or survival of offspring were observed, however further analysis of tissue samples at various growth stages suggests that TBECH may be metabolized too quickly to cause any long-term effects.

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PERSISTENT ORGANIC POLLUTANTS (POPs) IN THE NE PACIFIC OCEAN: LESSONS LEARNED FROM ENDANGERED KILLER WHALES

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Ten years ago, we reported that the killer whales plying the transboundary waters of British Columbia and Washington were among the most polychlorinated biphenyl (PCB) contaminated marine mammals in the world. Since then, we have learned much about the reasons underlying this heavy contamination. While the biomagnification of persistent organic pollutants (POPs) in marine food webs has been well described, killer whales appear to be especially vulnerable as a result of a very high trophic level, a relative inability to metabolize PCBs, and very long lives. Recent work in our laboratory suggests that PCBs are affecting the expression of a number of genomics endpoints, including the aryl hydrocarbon receptor (AhR), estrogen receptor α (ERα), thyroid hormone receptor α (TRα), and interleukin 10 (IL-10). This suggests that the endocrine, immune and reproductive systems of individuals are affected by exposure to PCBs and related compounds. While PCBs have declined up to four-fold in killer whales since 1972, this decline has slowed over the past decade, and structurally-related compounds such as PBDEs have increased. These findings highlight the failure of chemical regulations to adequately predict the fate and effects of POPs in the environment, and protect high trophic species from injury. As southern resident killer whales are the subject of dedicated recovery efforts under endangered species legislation in both Canada and the USA, these findings have generated strategies that reduce inputs of persistent environmental contaminants into killer whale habitat. Recent regulation of PBDEs in Canada and withdrawal from the US market appear to be leading to declines in the concentration of these flame retardants in biota in coastal BC and Washington. Ocean disposal practices have been modified in Canada to reduce the risk of contamination of killer whale food webs. Remediation of contaminated sites is targeting legacy PCB sources in both Canada and the USA. Sewage treatment practices are being scrutinized in an effort to reduce the release of a variety of contaminants of concern. Collectively, these mitigation efforts will reduce the risk of exposure and effect in the region's killer whales and increase the likelihood of population recovery.

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CONTAMINANTS AND IMMUNOTOXICITY IN PINNIPEDS AND CETACEANS

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The oceans continue to serve as a sink for a myriad of environmental contaminants of urban, industrial and agricultural origin. The highest concentrations of many contaminants are often found in estuaries and coastal areas, the habitats for a number of marine mammal species. High trophic level marine mammals are particularly vulnerable to contamination by persistent environmental contaminants, in some cases leading to adverse health effects such as immunotoxicity, endocrine disruption, reproductive impairment, and developmental abnormalities. Designed to fend off invasion by foreign pathogens and to prevent the growth of malignant cells by a variety of effector cells, molecules and chemical messengers, the immune system can be impaired by exposure to a variety of environmental contaminants. Studies in laboratory animals have documented the causal links between environmental contaminants and the reduction of host resistance to infections or the development of tumours. With marine mammals, logistical, technical and ethical constraints limit such mechanistic studies, but *in vitro* exposures represent an alternative and less invasive approach. Moreover, *in vitro* approaches enable a discrimination between direct and indirect effects on immune cells, and the investigation of some of the mechanisms of toxicity at cellular and molecular levels. Most of the immune functions characterized in marine mammals have been demonstrated to be sensitive to *in vitro* exposures to environmentally relevant doses of the major environmental contaminants measured in marine mammal tissues. This presentation will provide a brief history of marine mammal immunotoxicology and an overview of immunotoxic effects reported.

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TRANSFER OF PCBS AND PBDES FROM MOTHER TO FETUS IN BELUGA WHALES (DELPHINAPTERUS LEUCAS) FROM THE WESTERN CANADIAN ARCTIC

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Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are ubiquitous in the environment and have a well-documented propensity to biomagnify in marine food webs. Exposure to these toxic contaminants has been linked to immune dysfunction, neurotoxicity and disruption of endocrine and reproductive systems in marine mammals. Health threats become particularly concerning in neonates as they are exposed to high levels of these compounds through reproductive offloading from their mothers. Contaminants are transferred from female to offspring transplacentally during gestation and via milk ingestion during lactation. Lactational transfer in mammals during nursing is well described and accounts for more than 80% of the total reproductive transfer; however in utero transfer has rarely been documented. In this study, the placental transfer of PCBs and PBDE was characterized in two western Beaufort Sea mother-fetus beluga (*Delphinapterus leucas*) pairs. These arctic beluga whales transferred, on average, 11.4% (7.5 mg) of their PCBs 11.1% (0.1 mg) of their PBDEs to their near-term fetuses. A single physico-chemical parameter, Log K_{OW}, largely explained the kinetics of this transplacental transfer for PCBs (r^2 = 0.79, p < 0.00001) and PBDEs (r^2 = 0.37, p = 0.007), with congeners having a Log K_{OW} < 6.5 preferentially transferred to the fetus. Blubber concentrations of 257 ng/g lipid weight (lw) PCBs and 3.8 ng/g lw PBDEs in beluga fetuses highlights the exposure to endocrine disrupting compounds during a critical developmental stage. The implications of detecting these levels of legacy PCBs and the flame retardant PBDEs in unborn Arctic beluga are unclear.

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AN ASSESSMENT OF EXPOSURE AND EFFECTS OF PERSISTENT CONTAMINANTS IN RIVER OTTERS (*LONTRA CANADENSIS*) IN VICTORIA HARBOUR , BRITISH COLUMBIA

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Monitoring contamination in wild otter populations has proven challenging due to their elusive nature and the invasive sampling techniques traditionally involved (e.g. trapping and tissue sampling). We recently reported that scats collected from river otter latrines in Victoria Harbour, BC, Canada contained levels of PCBs that exceeded 9 mg/kg lipid, a published criteria for reproductive impairment in mustelids. Subsequently, we applied an individual based approach by combining fecal DNA genotyping with contaminant and diet analysis of individual river otter scat samples from the Victoria Harbour population. That revealed variation in individual contaminant exposure along a gradient from industrialized to natural nearshore environments. Genetic data also indicated small scale population structuring, suggesting that only certain otters (one subpopulation) were exposed to contaminants at levels of concern. This study combined live animal sampling and radio-telemetry with non-invasive scat sampling. The objective was to evaluate the reliability of individual, population and contaminant data derived from river otter scat. This approach was also well suited for defining individual home ranges and characterizing contaminant exposure in this population. Individual river otters were radio-tracked to investigate home range, distribution and movement patterns. Telemetry data revealed that individual river otters inhabit home ranges that span approximately 5kms of coastline. Movement patterns indicate there is limited if any mixing between the proposed subpopulations and that certain otters inhabit the contaminated areas year round. Understanding the movement patterns and ranges of these individuals will help to further investigate bioaccumulation of contaminants from prey to predator, as well as any physiological effects from this exposure. The data derived from feces will be compared to river otter blood and tissue data to investigate bioaccumulation of contaminants from prey to predator, as well as any physiological effects from this exposure.

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Special Session Poster Presentations

PERSISTENT ORGANIC POLLUTANT CONCENTRATIONS IN URBAN RAPTORS OF BRITISH COLUMBIA

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The Cooper's hawk, *Accipiter cooperii* and the peregrine falcon, *Falco peregrinus*, are raptorial birds that use urbanized habitats. Cooper's hawks nest in city parks and along treed streets, while peregrines use high rise buildings, bridges and similar structures. Both species are top predators feeding mainly on birds, with populations remaining throughout the year in many cities, attracted by pigeons, starlings and common bird feeder species. Past studies linked population decline of falcon and accipiter species with exposure to legacy POPs, particularly DDE and dieldrin. There is recent data from both salvaged eggs and tissues of carcasses showing the continued accumulation of toxicologically significant concentrations of both legacy POPs and PBDEs. Therefore, we analyzed liver tissue from 21 Cooper's Hawks and 6 peregrine falcons found dead, 1999 – 2009, in the urbanized Lower Mainland area and compared them to samples from southeast Vancouver Island and the Okanagan Valley regions of British Columbia. Carcasses were selected only if body condition was good and the diagnosis of death was trauma. Hepatic ΣPBDE concentrations in 13 Cooper's hawks from the Lower Mainland were very variable, ranging from 30 to 4820 ng/g ww with a geomean of 450 ng/g ww. In the same sample, geomean ΣPCBs were 451 ng/g (range 40.5 to 4280 ng/g), and of DDE, 1280 ng/g (range 231 – 10,400 ng/g). In comparison, in the less urbanized Okanagan Valley, mean ΣPBDEs in three Cooper's hawk livers were only 17.2 ng/g (range 3.2 -133 ng/g), while DDE was higher, 1840 ng/g (range 441 to 3800 ng/g) consistent with heavy past use of DDT in that region. Results for peregrine falcons were comparable for averages and ranges.

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A THREE-GENERATIONAL STUDY OF *IN OVO* EXPOSURE TO PBDE-99 IN ZEBRA FINCH (*TAENIOPYGIA GUTTATA*)

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Egg injection was validated for studies of *in ovo* exposure to xenobiotics in the zebra finch (*Taeniopygia guttata*). *In ovo* injection was used to test the long-term, inter-generational effects of embryonic exposure to the polybrominated diphenyl ether PBDE-99. Eggs were dosed at 10, 100 and 1000 ng/egg and chicks, their offspring and grand-offspring were followed (three generations). *In ovo* PBDE exposure did not affect hatching success, chick growth, thyroid hormone levels or hematology (measured at 30 and 90 days of age). However, there were effects of PBDE treatment on adult phenotype of *in ovo* exposed bird: reduced clutch size, longer laying intervals. Second generation chicks of PBDE-exposed parents had decreased growth, but there were no longer-term effects on adult reproductive phenotype of second-generation offspring, or growth of their (third-generation) offspring.

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Pacific Northwest Chapter

Society of Environmental Toxicology and Chemistry

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21st Annual Meeting

Platform Presentation Abstracts

EVALUATING THE EFFICACY OF THREE PARALLEL SECONDARY MUNICIPAL WASTEWATER TREATMENT SYSTEMS WITH TRANSCRIPTOMIC ENDPOINTS OF A BULLFROG C-FIN ASSAY

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The marked increase in the use of pharmaceuticals and personal care products (PPCPs) is suspect in the endocrine disrupting effects seen in aquatic ecosystems. PPCPs represent a significant challenge to municipal wastewater treatment (WWT) facilities, as common methods of WWT were not specifically designed for effective removal of emerging contaminants. In addition to estrogenic effects of PPCPs, thyroid hormone (TH) effects, although more broad-ranging than sex hormones, are often overlooked in ecotoxicology. Frogs are environmental sentinels as they are highly sensitive to environmental changes and hormonal effects. The ability of the frog to respond to exogenous estrogens is superseded only by a dramatic response to TH. Metamorphosis from tadpole to adult depends solely on TH. We have developed a method to rapidly screen for endocrine disrupting effects of wastewaters in *Rana catesbeiana* tadpoles using the cultured tail fin (C-fin) assay. The C-fin assay uses eight, functionally athyroid, premetamorphic tadpoles. Twelve 4-mm tail fin biopsies are collected from each tadpole and are placed into tissue culture wells containing serum-free medium. Each biopsy is then exposed to different treatment conditions of various effluent concentrations in the presence or absence of TH or estrogen. After 48 h, the biopsies are collected, the RNA is isolated, and quantitative real time polymerase chain reaction (QPCR) is performed. Transcripts encoding proteins in hormone- and stress-signaling pathways are used as the first indicators of endocrine perturbations. The repeated measures design of the C-fin assay allows for screening of multiple conditions in one individual while maintaining biological variation and complex tissue structure. We are using the C-fin assay to compare, in parallel, three common secondary WWT systems receiving the same real municipal influent. Results indicate differential ability of these effluents to perturb gene expression.

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REGULATION OF ESTROGEN- AND ANDROGEN-RELATED GENES IN DEVELOPING FROGS: IMPLICATIONS FOR ENDOCRINE DISRUPTION

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There is now a consensus that amphibian populations have been declining since the 1960s. Endocrine disrupting chemicals potentially impair tadpole development and contribute to these declines, such as by disrupting sexual development through estrogen- (aromatase, cyp19, and estrogen receptors, er) and androgen- (5α-reductase, srd5α, and androgen receptor, ar) related genes. In anurans, sexual development may be especially complicated by the involvement of the thyroid hormone system, which can additionally be compromised by endocrine disruptors. We examined brain and gonad mRNA developmental profiles for genes related to sexual development (*cyp19*, *erα*, *erβ*, *srd5α1*, *srd5α2*, and *ar*) in three species of frogs, the Neotropical Túngara frog *Physalaemus pustulosus*, the Western clawed frog *Silurana tropicalis*, and the Northern leopard frog *Rana pipiens*, and then examined these genes' expression patterns after thyroid hormone exposure. We first established that all transcripts other than *sdr5α2*, were detected in the brain and gonad from premetamorphosis to climax, and for each target, the profile during development was similar between the three species. We then exposed prometamorphic tadpoles of each species to thyroid hormone (concentrations of 0.5, 5, and 50 nM for 48 h). Exposure to thyroid hormone resulted in different patterns of mRNA levels in each species: in *S. tropicalis*, the androgen-related genes, *srd5α* and *ar* increased, while in *P. pustulosus* and *R. pipiens*, the estrogen-related gene *cyp19* decreased. While the specific expression patterns differ between species after thyroid hormone treatment, stimulating androgen-related genes or decreasing estrogen-related genes might have the common outcome of affecting the balance of sex steroids and provides a potential mechanism of the masculinising effect of thyroid hormone in tadpoles. The interactions between estrogen, androgen, and thyroid hormone pathways are therefore important for normal tadpole development and along with species differences should be taken into account when examining endocrine disruption in amphibians.

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CYP2B6 IS THE ONLY CYP ENZYME RESPONSIBLE FOR THE OXIDATIVE METABOLISM OF BDE-99 BY HUMAN LIVER MICROSOMES

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The present study was conducted to assess if human *in vitro* oxidative metabolism of 2,2',4,4',5-pentabomodiphenyl ether (BDE-99) could be a key determinant of the much lower concentration of BDE-99 than 2,2',4,4'-tetrabomodiphenyl ether (BDE-47) in human samples. The *in vitro* oxidative metabolism of BDE-99 was assessed using pooled and single donor human liver microsomes (HLM), a panel of human recombinant cytochrome P450 (rCYP) enzymes, and a CYP2B6-specific antibody. The hydroxylated metabolites formed were quantified by a liquid chromatography/tandem mass spectrometry method. In HLM incubations, three major (2,4,5-tribromophenol (2,4,5-TBP), 5'-OH-BDE-99 and 4'-OH-BDE-101) and three minor (4-OH-BDE-90, 6'-OH-BDE-99, and 2-OH-BDE-123) BDE-99 hydroxylated metabolites were formed and identified using authentic standards. Four unidentified OH-penta-BDEs and one di-OH-BDE were also formed. A combined approach using a panel of human rCYPs, pooled HLM incubated with BDE-99 and an anti-human CYP2B6 specific antibody, and a panel of single donor HLM showing a wide range of CYP2B6 levels consistently showed that, *in vitro*, CYP2B6 is the only CYP enzyme active for BDE-99 oxidative metabolism and that CYP2B6 is responsible for the formation of all the eleven hydroxylated metabolites produced incubating HLM with BDE-99. Collectively, the results show that in HLM CYP2B6 is the only CYP enzyme responsible for BDE-99 oxidative metabolism, which is likely an important determinant for the bioaccumulation of BDE-99 in humans because of the high number and rates of hydroxylated metabolites formed. Formation of BDE-99 hydroxylated metabolites is also of toxicological concern as recent *in vitro* studies shown that several hydroxylated PBDEs including some of the human hepatic BDE-99 metabolites identified in the present study inhibit endogenous metabolism of thyroid and steroid hormones more potently than the parent PBDEs.

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INTERACTIONS OF HUMIC AND FULVIC ACIDS WITH SILVER NANOPARTICLES AND THE RESULTANT TOXICITY TO *CHLAMYDOMONAS REINHARDTII*

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Engineered nanoparticles (ENP) are used in a variety of consumer products, with over 1300 manufacturer-identified products in 2012 and an estimated 3400 products by 2020. Silver nanoparticles (AgNPs) constitute the largest percentage of ENPs in these consumer products. Demonstrated release pathways of the AgNPs into aquatic environments exist, but the hazard of these releases are poorly understood and predictions of hazards are difficult given the chemical complexities of AgNPs. It is generally accepted that the free ion form of most metals is the toxic form, but this does not consider the effect of the nanoparticle itself; with AgNPs in solution, the Ag can exist as both elemental silver and Ag^+. When natural organic matter (NOM) is considered, NOM may complex the Ag^+ or interact with the AgNP, potentially affecting the rate of Ag oxidation. Finally, many AgNPs have capping agents, which may themselves alter the chemical fate, transport, and toxicity. The goals of this study were to evaluate the chemical complexation and toxicity of Citrate, CO_3, and PVP capped AgNPs and $AgNO_3$ in MOPS buffer only and in the presence of humic and fulvic acids (representative of NOM). Polymer based ISEs were used to determine the concentration of Ag^+ for each AgNP in the presence and absence of NOM. The photosynthetic effects (IC50s) to *Chlamydomonas reinherdtii* were also determined in MOPS only or with NOM. The toxicity ranking of the capped AgNPs in MOPS only was PVP > Citrate>CO_3, which can in part be explained by the measured Ag^+. The AgNPs in the presence of the NOM had less measured Ag^+ than the AgNP in MOPs alone, but toxicity (as Ag^+) was not affected by NOM in the CO_3 and PVP coated nanoparticles. Toxicity (as Ag^+) did decrease in the presence of NOM for $AgNO_3$ and the Citrate coated AgNPs.

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THE EFFECT OF SULPHATE ON THE AQUATIC AND DIETARY UPTAKE OF SELENIUM IN THE WATER FLEA, *DAPHNIA MAGNA*

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Due to similar chemical characteristics, biochemical systems may substitute excess selenium for sulfur in protein structure (Presser and Luoma 2010, Stadtman, 1974). The excess integration of selenium in protein is believed to be the main mechanism underlying selenium toxicity. To further an understanding of the sulphur-selenium relationship, we explored the role of sulphate in the aquatic and dietary uptake of selenium in the freshwater water flea, *Daphnia magna*. An algae, *Pseudokirchneriella subcapitata*, was exposed to a range of sodium selenate concentrations (0, 10, 20, 40 µg Se/L) in waters with 3 treatments of sulphate (80, 160, 320 mg SO_4 /L). After 7 days, the algae were collected for use in feeding trials with *Daphnia*. *Daphnia* were provided the exposed algae either in the presence of the same aquatic selenium and sulphate concentrations or in sulphate treatment waters only. *Daphnia* were exposed for 21 days after which tissue was collected for selenium accumulation analysis. Analysis of *P. subcapitata* demonstrated an increase in selenium accumulation with increasing aquatic selenium, however, the quantity of selenium decreased with increasing sulphate concentrations. *Daphnia* also exhibited decreasing selenium accumulation with increasing sulphate concentration in the water. This study demonstrates that sulphur, in the form of sulphate, decreases the total selenium uptake in *D. magna*.

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ADVANCES IN THE USE OF POPULATION MODELS FOR ECOLOGICAL RISK ASSESSMENT OF PESTICIDES

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There has been increasing interest in the use of population models to improve the ecological risk assessment process of pesticides. A number of advances have occurred over the past several years that may make use of models more acceptable to risk assessors. In this talk I will go over the ecological risk assessment process for pesticides as it is now practiced, show what population modeling can do to improve the process, and go over several new advances in this area.

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USING SPATIALLY EXPLICIT DATA AND MODELING TO INFORM ECOLOGICAL RISK ASSESSMENT FOR A PROPOSED PIT MINE EXPANSION

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A screening level ecological risk assessment (SLERA) was conducted for the proposed Vista Phase 7 Pit Lake, an expansion of an existing Pit Mine owned and operated by Newmont Mining Corporation in northwestern Nevada. As is typical for SLERAs, very conservative assumptions for both toxicity and exposure were initially used to evaluate the potential for unacceptable risk. As a result, the initial screening approach provided minimal information to refine the list of chemicals of interest needed to focus risk management and reduction strategies. To provide a more complete evaluation of potential risk, a refined screen was conducted using expanded and spatially explicit modeled and empirical site data for multiple media including soil and expected future sediment and surface water conditions. These data were used in conjunction with an expanded list of toxicity values and exposure pathway models to evaluate risks to ecological receptors at the site. Multiple exposure scenarios were used to evaluate both baseline risks and potential mitigation of risks using overlay material to reduce exposure to areas with higher levels of chemicals of interest. The results of exposure modeling indicated that a handful of metals, including aluminum, antimony, arsenic and mercury, showed potential for risk to some aquatic ecological receptors. However, an evaluation of geochemical conditions indicated that expected conditions at the future Pit Lake are likely to limit bioavailability to ecological receptors, reducing the potential for unacceptable risk. The results of this SLERA illustrates the importance, even at a screening level, of a robust, spatially explicit data set, an in-depth understanding of site-specific geochemistry, and consideration of site management alternatives that may be available, in order to develop an accurate understanding of the potential for unacceptable ecological risk.

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REGIONAL RISK ASSESSMENT OF THE PUYALLUP RIVER WATERSHED AND LOW IMPACT DEVELOPMENT (LID) TO MEET MANAGEMENT GOALS

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Relative risk assessment is a tool to calculate the likelihood of effects to endpoints when multiple stressors occur in complex ecological systems. Here we use Bayesian network (BN) models derived from the relative risk assessment framework. We plan to create a model that can evaluate the risk Low Impact Development is able to reduce in the Puyallup River Watershed for ecological endpoints. The BN structure comprises of three tiers of nodes: landscape stressors, habitats, and ecological endpoints of interest to local managers. Each tier links nodes to lower-tiered nodes when spatial and ecological relationships exist. Four potential discrete states exist for all parameters: zero, low, medium, and high with numeric scores of 0, 2, 4, and 6 respectively. As a first step in model creation, here we present conceptual models showing causal pathways between stressors and endpoints. Conceptual models for the endpoints of water quality, salmon, and flood control have been created. The next step will be to create a BN structure for each endpoint. The adaptability of using BNs for a relative risk assessment provides opportunity for the model created to be adapted for other watersheds in the Puget Sound region with the same restoration goals. If our model finds Low Impact Development is able to reduce risk, the goal of this model will be to create a relative risk model that may be used in future watershed restoration to determine what types and where Low Impact Development should be implemented.

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AN EXPLORATION OF EXPOSURE SCENARIOS FOR ANADROMOUS FISH

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In 2011, as part of an update of state water quality standards (WQS) for protection of human health, Oregon adopted a fish consumption rate of 175 g/day for freshwater and estuarine finfish and shellfish, including anadromous species. WQS whose derivation is based, in part, on anadromous fish, create the expectation that implementation of these WQS will lead to lower contaminant levels in returning adult fish. Whether this expectation can be met is likely a function of where and when such fish are exposed. This study examined 16 different exposure scenarios with bioenergetics and toxicokinetic models to identify those where WQS might be effective in reducing polychlorinated biphenyls (PCBs) - a representative bioaccumulative contaminant - in returning adult Fall chinook salmon - a representative salmonid. Model estimates of tissue concentrations and body burdens in juveniles and adults were corroborated with observations reported in the literature. Model results suggest that WQS may affect limited ($\leq \approx 2\times$) reductions in PCB levels in adults who were resident in a confined marine water body or who transited a highly contaminated estuary as out-migrating juveniles. In all other scenarios examined, WQS would have little effect on PCB levels in returning adults. While the results of any modeling study must be interpreted with caution, and these are not necessarily applicable to all salmonid species, they do suggest that the ability of WQS to meet the expectation of reducing contaminant loadings in anadromous species is limited.

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DEVELOPMENT OF A SPATIALLY EXPLICIT POPULATION MODEL FOR PCB EXPOSED MINK

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Toxicological effects data indicate the potential for large reductions in reproductive measures for American mink (*Mustella vison*) at environmentally relevant polychlorinated biphenyl (PCB) doses. Given their sensitivity, mink often drive risks at PCB contaminated sites. However, when we predict unacceptable risk based on comparison of average doses within a contaminated site to organism-level effects measures, the exposed population may or may not be affected. Population-level impacts depend on the dynamics of birth, dispersal and stage-specific survival of individuals throughout a complex landscape where habitat quality and contaminant concentrations vary. To make more realistic estimates of risks to PCB exposed mink populations, we developed a spatially explicit population model incorporating four important sources of uncertainty: a probabilistic exposure model, a dose-response effects model, a spatially explicit habitat model, and a spatially explicit population model. Model development and uncertainties will be discussed.

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BIOLOGICAL EFFECTIVENESS OF GREEN STORMWATER INFRASTRUCTURE: ASSESSING SUBLETHAL IMPACTS OF URBAN STORMWATER RUNOFF TO DEVELOPING FISH AND INVERTEBRATES

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Urban stormwater runoff contains a complex mixture of contaminants, including dissolved metals and polyaromatic hydrocarbons (PAHs), which can be acutely lethal to developing fish and invertebrates. Green stormwater infrastructure (GSI) can reduce the impact of stormwater runoff to aquatic habitats by reducing flows and pollutant concentrations. However, the biological effectiveness of GSI is not known; will stormwater runoff treated by GSI elicit toxic effects on aquatic biota? Which GSI methods are most biologically effective? In order to answer these questions, we must have a set of assessment tools that are relevant to the toxic impacts generated by exposure to stormwater runoff. Mechanisms of mortality and potential sublethal effects of stormwater runoff are currently poorly understood. We have been focusing on building a toolbox for assessing the biological effectiveness of GSI at reducing the toxicity of stormwater runoff. Runoff from an urban highway affected survival of developing fish and invertebrates. Surviving fish in runoff samples commonly displayed a suite of symptoms including hypophthalmia, jaw deformations, pericardial edema and cardiac abnormalities, as well as reduced growth and delayed swim bladder inflation. These symptoms are similar to those caused by sublethal exposure to petrogenic PAHs, such as those found in weathered crude oil. Recent findings will be presented and discussed.

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DEVELOPMENT OF A NEW SEDIMENT CHEMISTRY STATUS AND TRENDS INDEX FOR ENVIRONMENTAL MANAGEMENT IN PUGET SOUND, WA

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Status and trends sediment quality data have been collected annually in Puget Sound since 1989 by the Washington State Department of Ecology (Ecology) for the Puget Sound Ecosystem Monitoring Program. These data include measures of chemical contaminant levels, laboratory tests of sediment toxicity, and the condition of invertebrate assemblages living within the sediments. Various methods have been developed over time to summarize and report these three sets of sediment data both separately and combined. In 2007, Washington law makers passed legislation establishing the Puget Sound Partnership (PSP) as the state agency responsible for protecting the health of Puget Sound and improving its condition by 2020. To help recognize success, legislative requirements included adoption of ecosystem recovery indicators and targets by which condition and change over time can be measured. The PSP subsequently adopted a suite of "Dashboard of Vital Signs" indicators, including a Sediment Quality Index based on Ecology's data (http://www.psp.wa.gov/vitalsigns/index.php). Ecology's Marine Sediment Monitoring Team has recently revised its methods for reporting sediment quality condition and change over time as a new Sediment Chemistry Index (SCI), Sediment Toxicity Index, Sediment Benthos Index, and a combined Sediment Quality Triad Index. Methods for calculating the SCI are presented, along with a preview of the graphic displays of these new indicators.

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TOWARDS ECOSYSTEM BASED SEDIMENT QUALITY CRITERIA

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Sediment quality criteria for contaminants are widely used by government agencies and consultants for a range of purposes, including conducting risk assessments, setting goals for remediation and setting permits for ocean disposal of contaminated sediments. In most cases, the sediment quality guidelines are based on toxicity information for benthic invertebrates and hence aim to protect benthic invertebrate populations. The sediment quality criteria are in most cases not suitable to protect other organisms such as fish, crabs, marine mammals, birds and human populations who may consume various organisms through fishing, crabbing and other forms of hunting. The objective of this study is to develop and evaluate methods for developing sediment quality criteria for contaminants that have greater ecological and human health relevance. We demonstrate empirical methods and food-web bioaccumulation models to develop such ecosystem guidelines for PCBs in the Pacific North West. The findings illustrate that current sediment quality guidelines fail to protect the majority of Orca whale, Stellar sea lion and harbor seal populations. Current guidelines also do not ensure that fish and shellfish populations are below Tissue Residue Guidelines for human consumption. Sediment quality guidelines calculated from relevant endpoints for human health, marine mammal population health and to meet Chinook salmon tissue residue guidelines ranged from 0.002 µg/kg dw to 2.7 µg/kg dw – substantially lower than the current B.C. SQG of 20 µg/kg dw.

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OCCURRENCE AND BIODEGRADATION OF A NOVEL PFOS-PRECURSOR, THE PERFLUOROOCTANE SULFONAMIDO ETHANOL-BASED PHOSPHATE DIESTER (SAM-PAP) IN MARINE SEDIMENTS

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Perfluorooctane sulfonate (PFOS) is a widespread global environmental contaminant, yet the sources of this compound have not yet been fully elucidated. Among the potential sources of PFOS are numerous precursor compounds (i.e. N-alkyl substituted perfluorooctane sulfonamides, "PreFOS"), some of which have been detected in the environment. N-ethyl perfluorooctane sulfonamido ethanol-based phosphate mono- di- and tri-esters (SAmPAPs) are one class of PreFOS used historically in the food paper and packaging industry and are currently manufactured in Asia. The introduction of SAmPAPs in the 1970s and their subsequent phase-out in North America in 2002 correspond with temporal trends of PFOS in North American human sera over this time. While the presumed metabolites and biodegradation products of this compound have been reported, the environmental occurrence or stability of SAmPAPs remains unknown. Herein we report the first measurements of the Sam-PAP diester and its biodegradation potential in the environment. Water and sediment samples were collected from False Creek, a marine harbour in Vancouver, Canada. The SAmPAP diester was detected at concentrations ranging from 100-200 pg/g in sediments, which was similar to PFOS and other PreFOSs. A significant correlation (p<0.05) was observed between the concentration of the SAmPAP diester and its presumed degradation product, N-ethyl perfluorooctane sulfonamido acetate (NEtFOSAA). While the SAm-PAP diester was not detected in water, PFOS was observed at concentrations of up to 600 pg/L. Sediment biodegradation experiments involving the Sam-PAP diester indicated that this compound is highly recalcitrant to microbial degradation (no biodegradation was observed after 120 days). In contrast, N-ethyl perfluorooctane sulfonamido ethanol (a presumed metabolite of SAmPAP) readily degraded in False Creek sediments to produce a suite of perfluorooctane sulfonamido acetates, and PFOS. The persistence of SAmPAP suggests that this compound may be widely distributed in the environment beyond False Creek, and further investigations are warranted.

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INTRODUCTION TO THE CALCULATION OF RISKS DUE TO MERCURY AND OTHER STRESSORS TO MULTIPLE ENDPOINTS FOR THE SOUTH RIVER AND UPPER SHENANDOAH RIVER, VIRGINIA AND THE PROJECTED OUTCOMES OF DIFFERENT MANAGEMENT SCENARIOS.

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A cumulative integrated risk assessment has been performed for the South River from the area upstream of Waynesboro VA to the uppermost part of the Shenandoah River. The area is a site of historic Hg contamination from synthetic fiber production in Waynesboro. Six risk regions have been delineated. Other sources of stressors include urban and agricultural runoff, channelization, erosion, and contaminated sediments and biota. In this presentation the current iteration of the relative risk model (RRM) incorporating the hierarchical patch dynamics paradigm was used to construct a conceptual model, designate risk regions, create a ranking system and calculating risks to the stakeholder derived endpoints and impacts. The Bayesian network (BN) derivative of the RRM has also been used and is reported in other presentations. Atypically, the current source of the Hg contamination is the environment itself. The warm water fish species have been found to have consistently higher tissue concentrations downstream of the original source. Nutrients from upstream of Waynesboro also may be contributing to risk to a variety of endpoints. Our results demonstrate that the risk is unevenly distributed along the course of the watersheds. Ecological services are at highest risk throughout the study area. Mercury is the stressor contributing the highest risk but other stressors are large contributors. Explorations of management scenarios that reduce nutrients or remove Hg are being conducted. A nutrient reduction of 50 percent does reduce risk is the higher risk areas, but only in the range of 15-20 percent. Lower risk areas are minimally affected. The RRM has proven to be a useful approach to evaluating management scenarios. We will demonstrate that no magic single solution exists for the study area and that a watershed management plan that addresses multiple stressors is necessary to reduce risk.

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UBIQUITOUS, FLYING OUTLIERS – SKEWNESS AND ITS RAMIFICATIONS FOR BSAFS, BACKGROUND THRESHOLD VALUES, AND SITE CONCEPTUAL MODELS

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How many times have you plotted tissue vs. sediment concentrations and had one or two sediment and/or tissue values flying an order of magnitude beyond all the other data in the data set? How many univariate background or site sediment and tissue data empirical frequency distributions (EDFS) have you looked at with one or two or even five such values? It has long been recognized that chemical concentrations, whether in the sediment or in tissues, tend to be skewed to the right….often with just a few high values flying beyond the majority of lower values. Sometimes this "right skewness" can be made to look more "normal" by a data transformation, but often, the data are so skewed that no transformation seems to adequately rescale the large separation between the highest values and the bulk of the data. Though the USEPA has provided a statistical package (ProUCL) to compute valid percentiles, confidence intervals, and differences between means for highly skewed distributions, the desire to label high values outliers and eliminate them from a data set is still strong since they create such uncertainty in the statistics used to determine areas for environmental cleanup. The goal of this talk is to begin to develop a context and vocabulary for addressing these high values – relatively rare within any individual data set, but so ubiquitously present in all data sets – that reduces arm-waving and increases understanding. We will discuss how outlier tests, nonparametric statistical methods, laboratory measurement and field duplicate error, and valid sampling designs can help evaluate the true frequency and magnitude of these data in a given population. In any project, these types of discussions can contribute to refinement of the site conceptual model, cost-benefit analyses of the need for additional data, and, most importantly, shared trust in the data.

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Poster Presentation Abstracts

THE USE OF BAYESIAN NETWORK MODELS TO EVALUATE ECOLOGICAL RISK FROM HG CONTAMINATION IN THE SOUTH RIVER, VA

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Despite the time that has elapsed since mercury was released into the South River in northwestern Virginia, mercury concentrations in the river, fish and wildlife remain high. We used Bayesian network models to evaluate the potential impacts of mercury and other stressors to fish and wildlife in the South River watersheds. We evaluated risk to several organisms of concern, including smallmouth bass (omnivorous fish), kingfishers (piscivorous bird), and Carolina wren (insectivorous bird). For each organism we evaluated the likelihood of ecological impacts resulting from methyl mercury body burden as well as other potential ecological stressors, such as microclimate conditions, habitat suitability, and other contaminants in the environment. In general, Bayesian network models are well-suited for ecological risk assessments because: 1) the structure reflects the causal relationships between stressors, organisms, and potential impacts, 2) uncertainty is explicitly incorporated, and 3) the model can also be used as a risk management and communication tool. Our risk assessment models indicated that mercury contamination is not the only stressor impacting fish and birds in the South River watersheds and efforts to protect species of concern will require more than removing mercury from the river.

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AN ASSESSMENT OF RISK TO WATER QUALITY AND ECOLOGICAL SERVICES FOR THE SOUTH RIVER, VA

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Like most river systems, the South River and its watersheds support various land and recreational uses. Predominant land uses are agricultural areas, mainly pastures, and low intensity development. The river receives effluent from light industrial plants and urban run-off, and also supports a large water treatment facility in Waynesboro, VA. The South River is listed by US EPA as an impaired river based on mercury contamination, fecal coliform bacteria, and assessments of benthic macroinvertebrate (BMI) assemblages. Communities along the river use it extensively for recreation; the most popular uses are fishing, boating and swimming. Improving water quality and maintaining the river's ability to support recreational use are priorities for governmental and other stakeholder groups. In response to stakeholder interests, we developed a Bayesian network model based on causal relationships between stream chemical and hydraulic parameters and ecological services. The model was parameterized using data from several sources: U.S. Geological Survey, Virginia Department of Environmental Quality, and the South River Science Team. Preliminary results suggest that BMI communities are most at risk of impacts from alterations to the river. The level of risk to BMI communities was influenced by changes in stream flow. Swimming was the recreational use that had high risk, influenced by water temperature and stream flow conditions. In our model stream flow and temperature were expressed as the difference between current conditions and the long-term, seasonal average so that the model can be used to evaluate the impact of regional climate change scenarios on water quality standards and recreational uses. This approach is novel because it examines how alterations to stream conditions can impact ecological services that people value in a probabilistic fashion

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EVALUATION OF TOXICITY MODIFYING FACTORS FOR FLUORIDE USING *HYALELLA AZTECA* AND RAINBOW TROUT

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Previous studies investigating the toxicity of fluoride have suggested that both hardness and chloride appear to modify its toxicity. This study involved an evaluation of hardness, alkalinity and chloride as potential toxicity modifying factors for fluoride. Testing was conducted using acute exposures (96 hr) to *Hyalella azteca* and rainbow trout (*Oncorhynchus mykiss*). Sodium fluoride was added into laboratory-prepared waters with a range of chloride, alkalinity and hardness concentrations. The test series was designed to evaluate the effect of independent and correlated variations of the parameters under investigation (hardness, alkalinity and chloride) on modifying the toxicity of fluoride. In the *H. azteca* tests, when chloride concentrations were held constant but hardness and alkalinity concentrations increased and co-varied, there was a minimal change in LC50 values. In all other scenarios that involved variations in chloride concentration, LC50 values increased with increasing concentrations of the evaluated factors. These results suggest that an increasing chloride concentration explains most of the decrease in fluoride toxicity, and is the primary toxicity modifying factor for this species. The relationships were less clear in the rainbow trout tests, although a similar overall pattern of results were observed. This suggests that for *H. azteca* as well as rainbow trout, chloride plays a key role in modifying the toxicity of fluoride.

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EXPLORING THE RELATIONSHIP BETWEEN THE BCF, BAF AND THE TMF

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There is a reasonable degree of consensus among bioaccumulation scientists on the use of Trophic Magnification Factors (TMF) as "conclusive" evidence of the bioaccumulative nature of chemicals in the environment. However, regulatory criteria for the determination of whether a substance is bioaccumulative mostly rely on the use of the Bioconcentration Factors (BCF), measured in laboratory tests. In this paper, we present the results from laboratory, field and modeling studies aimed at testing the hypothesis whether the BCF is a good predictor of the TMF. Our studies involved (i) the compilation of BCFs and BAFs for a range of potentially bioaccumulative substances; (ii) a field study of the food-web bioaccumulation of the same substances in Western Scheldt Estuarine food-web aimed at determining the TMF in aquatic food webs; and (iii) a modeling study aimed at formulating and exploring the relationship between the BCF and the TMF. We conclude that the BCF can be a useful predictor of the TMF for a range of chemicals with specific characteristics and under specific ecological conditions. We also conclude that there are two major types of "exceptions" where the BCF does not provide accurate information about the bioaccumulative nature of chemicals in the environment. The first exception deals with substances that show a relatively low BCF but have a high TMF. The second exception is for chemicals that exhibit a high BCF in laboratory tests, but which do not show biomagnification in food-webs and have a low TMF. Supported by model calculations, we propose a set of simple rules that may be useful in the interpretation of laboratory based bioconcentration factors in terms of their true bioaccumulative nature in the environment. The rules may be helpful to regulators involved in bioaccumulation assessments and to scientists that apply bioconcentration studies to study the bioaccumulative nature of chemicals in the environment.

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DETERMINING THE ADDITIVE, SYNERGISTIC OR ANTAGONISTIC EFFECTS OF CHEMICAL MIXTURES OF CARBARYL AND MALATHION ON *DAPHNIA MAGNA*

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The composition of stormwater is a mixture of different chemicals from different non-point sources. The continued urbanization of areas leads to more impervious surfaces that allows contaminates to be washed into nearby water ways. With the continued use and over use of pesticides the potential for mixtures of these chemicals from their over-application creates a need to understand the combined toxicity for the mixtures of chemicals and how they affect non-target organisms. For this experiment a 48 hour acute toxicity test will be conducted with analytical grade carbaryl with treatment concentrations ranging from 1.25-20.0 µg/L and analytical grade malathion between 0.5-8.0 µg/L using *Daphnia magna* as the test species. Mixture toxicity of the two chemicals was conducted using the treatment concentrations of carbaryl mixed with 1.0 µg/ L of malathion solution in order to look at the potential additive, synergistic or antagonist effects of mixtures of carbaryl and malathion to address the toxicity on non-target species.

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TOXICITY OF SILVER NANOPARTICLES WITH THREE CAPPING AGENTS (PVP, CITRATE, and CO_3): AN EVALUATION OF IONIC VERSUS TOTAL SILVER

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Silver nanoparticles (AgNP) are a useful bactericide with seemingly endless applications, however little is known of the mechanism for this toxicity. As the use of AgNP increases, so does the potential for environmental releases. Nanoparticles are incredibly complex molecules and the environmental impacts of these releases are poorly understood. Nanoparticle chemistry can be altered, for example, by capping them. This alters the behavior and utility of the materials in end-use products, and further complicates the understanding of fate, transport and toxicity of the materials. This study compared the toxicity of AgNP with three different capping agents (PVP, CO_3 and Citrate) and $AgNO_3$. The Microtox basic acute toxicity test was used to determine the IC50s. The percent Ag^+ was measured in each AgNP type with a polymer membrane Ion Selective Electrode. The IC50s were compared as total Ag and as Ag^+ to elucidate the toxic contribution of the nanoparticle itself versus the Ag^+. The toxicity ranking based on the IC50s, as total Ag, was $AgNO_3$ > AgNP PVP > AgNP Citrate > AgNP CO_3. When toxicity was expressed as Ag^+, however, there was no difference between the $AgNO_3$, AgNP PVP and AgNP Citrate, which indicates that the ionic form of the Ag is responsible for the observed toxicity. The AgNP CO_3, however, was less toxic than the $AgNO_3$. The mechanism for this decrease in toxicity is being investigated.

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USING A BAYESIAN NETWORK APPROACH TO EVALUATE THE EFFECTIVENESS OF LOW IMPACT DEVELOPMENT TECHNIQUES IN MANAGING RISK IN THE PUYALLUP RIVER WATERSHED

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Regional risk assessment is a framework used to calculate the likelihood of specified impacts to a set of identified endpoints in complex, ecological systems where multiple stressors are present. The area of interest is broken into risk regions based on habitat and land use. We will be evaluating the risk associated with various natural and anthropogenic stressors to stakeholder endpoints in the Puyallup River watershed, as well as the effectiveness of various low impact development practices in reducing risk to the endpoints. Bayesian networks (BNs) are a tool used to formulate the relative risk rankings for each region. The structure of BNs consists of the following three tiers of nodes: landscape stressors, habitats, and ecological endpoints of value to stakeholders. The nodes of each tier link are linked to lower nodes if ecological or spatial relationships exist. For each parameter, four potential discrete states exist: zero, low, medium, or high with the assigned numerical score of 0, 2, 4, and 6 respectively. Management strategies, including the implementation of low impact development practices, will be evaluated for effectiveness in reducing risk to the endpoints. Associated uncertainty will be explicitly stated. Management goals are derived from the Puget Sound Partnership's goals for restoration of the Puget Sound.

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DOES EPA METHOD 7471 ACCURATELY MEASURE MERCURY CONCENTRATIONS IN FISH?

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We compared the extraction efficiencies of the sample preparation methods described in EPA Methods 7471 and 1631 Appendix for total mercury in fish samples. Many labs use EPA 7471 – "Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)" – for the determination of mercury in biological samples, although it is only approved for "soils, sediments, bottom deposits, and sludge-type materials." EPA 1631 – "Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)" – is approved for the analysis of water samples, and has an appendix – "Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation" – that extends the method to tissue analysis. In a side-by-side analytical comparison on fish samples, we observed that the preparation method from EPA 7471 resulted in mercury concentrations 20% lower than the method from EPA 1631 Appendix, when all sample digestates were analyzed by the same CVAFS technique.

The sample preparation procedures described in EPA Methods 7471 and 1631 differ in the oxidation reagent used and the digestion parameters. We hypothesized that incomplete digestion, incomplete mercury oxidation, or a combination of both are the cause of the low recoveries associated with EPA 7471. EPA 7471 specifies a digestion time of 30 minutes and potassium permanganate oxidation, while EPA 1631 Appendix specifies a digestion time of at least four hours and bromine monochloride oxidation. To test for incomplete digestion, we prepared a set of samples and CRMs following the digestion protocol in EPA 7471, but with increased digestion times. To test for incomplete mercury oxidation, we prepared a parallel set of samples and CRMs following EPA 1631 Appendix, but using potassium permanganate as the oxidizing reagent instead of bromine monochloride. We conclude that EPA Method 7471 poses a risk of low-biased data for total mercury in biological tissues.

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INFLUENCE OF TEMPERATURE ON HEAVY METAL TOXICITY TO *LEMNA TURIONIFERA*

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At high concentrations, heavy metals cause deleterious effects to lipids and proteins in vascular plants, commonly resulting in reduced growth. In *Lemna turionifera* (duckweed) this may be a result of decreased gas exchange or photosynthesis inhibition. Effects of temperature and metals on frond growth, frond survival, total chlorophyll and bioaccumulation are currently being investigated through 7d static renewal tests using plants from a local pond that were cultured for several months in the laboratory. Test organisms were acclimated for two weeks at 20°C and 28°C. They are currently being exposed to silver, copper, or chromium at each temperature. Preliminary results support a temperature dependent difference for copper toxicity, although these results are being confirmed.

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THE ACTIVITY OF P-GLYCOPROTEIN IS NOT REGULATED BY OXIDATIVE STRESS IN ISOLATED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) HEPATOCYTES

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To investigate the hypothesis that oxidative stress (OS) can regulate P-glycoprotein (P-gp) function in isolated rainbow trout hepatocytes (*Oncorhynchus mykiss*), cell suspensions were exposed *in vitro* to the pro-oxidant treatments diethyl maleate (DEM) or hydrogen peroxide (H_2O_2). Cellular glutathione (GSH) concentrations were depleted following treatment with 2.5 mM DEM and increased by treatment with 2.0 mM GSH. P-gp activity was assessed by measuring the accumulation of the P-gp substrate rhodamine 123 following treatment with either H_2O_2, DEM or GSH. Initial rates of R123 accumulation by hepatocytes treated with 0.025 to 2.5 mM DEM, 0.02 to 2.0 mM GSH, or 3 to 1200 µM H_2O_2 were not significantly different from their respective controls. The findings of this study suggest that P-gp activity in rainbow trout hepatocytes is not acutely modulated by increases in reactive oxygen species or changes in GSH content *in vitro*.

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RAPID RISK ASSESSMENT: EXPLORATORY ANALYSES USING AN EXCEL-BASED ECO-RISK TOOL

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Ecological risk assessments (ERAs) are complex and can require a significant amount of time well after exposure point concentrations (EPCs) are known and toxicity reference values (TRVs) are selected. Decision-makers often want the results of a risk analysis early to help in site decision making. Understanding and interpreting the datasets early in the process helps decision-makers identify information gaps or areas of uncertainty, understand which receptors and exposure routes will be most crucial in ultimate site decisions, know which lines of evidence (LOEs) may drive risk, and understand the importance of site-specific exposure parameters. Windward Environmental LLC has developed an easy to use Microsoft Excel-based Eco-risk Tool that utilizes macros to conduct rapid ecological risk calculations to help answer these questions early in the risk assessment process. The Eco-risk Tool incorporates site-specific EPCs, a range of TRVs for sediment, and tissue-residue and dietary dose LOEs, as well as other input parameters such as diet assumptions, to deliver hazard quotients for each chemical of interest with an EPC and TRV. The most useful advantage of the Eco-risk Tool is that it allows responsible parties to modify the TRVs and exposure parameters (e.g., site use factors, variable spatial areas, ingestion rates, and diet assumptions) rapidly, and as many times as they wish, to determine the effect of changes in exposure parameters on hazard quotients. The Eco-risk Tool is easy to use, since most individuals in the risk assessment field are familiar with Excel; window pop-ups with dropdown menus allow the user to easily modify the input parameters, and hazard quotients for each chemical of interest can be exported into separate excel worksheets for further analytical purposes. Laptops will be available to demonstrate the ease of use and capabilities of the Eco-Risk Tool.

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THE ACUTE TOXICITY OF ANTIHISTAMINES TO SNAILS, *LYMNAEA STAGNALIS*

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The acute toxicity of two common antihistamines, diphenhydramine (DPH) and cetirizine hydrochloride (CZH), will be determined using aquatic snails. A range of concentrations will be tested based on previous work with *Daphnia magna* that determined the LC_{50} of DPH to be 0.12µg/L at a pH of 8.5 (1). Groups of five juvenile snails will be placed in freshwater made according to a US EPA preparation, (2) with DPH concentrations ranging from 0 µg/L to 0.20 µg/L at intervals of 0.04 µg/L. The same range will be used to determine the LC_{50} of CZH. Both trials will be performed at a pH of 8.5 and again at a pH of 6.8. A similar trial will then be conducted with a mixture of DPH and CZH. Each test exposure will be run for 96 hours.

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A MODEL FOR ASSESSING THE BIOACCUMULATION POTENTIAL OF METABOLIZABLE HYDROPHOBIC SUBSTANCES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The lack of methods to assess the extent of biotransformation of chemicals is one of the drawbacks of current methods for assessing the bioaccumulation potential of commercial compounds. As a result, there is considerable interest in the development of methods and protocols to determine metabolic transformation rates of compounds. In this study, a modelling approach will be applied to describe the effect of biotransformation on the bioaccumulation of hydrophobic organic chemicals (log Kow > 4.0) in rainbow trout (*O. mykiss*). This three compartment model describes the bioaccumulation behavior of various chemicals in the liver, gastrointestinal tract, and the carcass. A feeding study provided us measured chemical concentrations in these three compartments throughout an uptake and elimination phase. Using this information, we have found a good agreement between measured liver, gastrointestinal tract and carcass concentrations and those predicted with the model. With known biotransformation rates, it will be possible to apply this model to predict the bioaccumulation behavior of hydrophobic organic chemicals in fish, which will be a step towards advance our extrapolation of *in-vitro* biotransformation rates to *in-vivo*.

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INVESTIGATION INTO TOXICITY OF CHEMICAL MIXTURES INVOLVING ORGANOPHOSPHATE PESTICIDES: MALATHION AND DIAZINON TOXICITY TO *DAPHNIA MAGNA*

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Freshwater habitats in urban and agricultural regions are commonly found to contain chemical mixtures from several nonpoint runoff sources. The suburbanization of land use in close proximity to agricultural regions allows contaminants applied to be transported into the nearest waterway. The common over-application of pesticides by landscaping and agricultural practices causes excess toxicant to be washed away with runoff. Recent studies on mixture toxicity have concluded that single toxicant risk assessments may be underestimating toxicity of certain pesticides to aquatic organisms when mixing in aquatic environments. To manage the implicit risk it is critical to understand how individual chemical toxicity mixtures can affect the survival and behavior of non-target organisms. Organophosphate pesticides inhibit the activity of acetylcholinesterase (AChE) and can generate effects that can interfere with freshwater communities by alteration of lower trophic levels. Due to reported synergistic toxicity of organophosphate mixtures, it is critical to assess mixture toxicity. In this experiment 48-hour acute *Daphnia magna* toxicity tests were conducted using >98% analytical grade malathion and >98% analytical grade diazinon. The chemicals were tested at the following concentration ranges: malathion 0.70 – 11.20 µg/L and diazinon 0.37 – 6.00 µg/L. A mixture of malathion and diazinon also were tested to evaluate the potential additive, synergistic, or antagonistic toxicity: malathion 0.70 – 11.20 µg/L in the presence of 0.75 µg/L of diazinon. The survival and behavior data collected will be used to determine how the chemical mixture would change the dynamics of a *Daphnia magna* population.

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EFFECTS OF URBAN STORMWATER RUNOFF ON MACROINVERTEBRATE COMMUNITIES AND IMPLICATIONS FOR ENDANGERED SALMONIDS

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Numerous studies have documented declines in the diversity and abundance of macroinvertebrate communities in urban watersheds. However, the extent to which these declines are caused by non-point source pollution as opposed to physical habitat factors remains unclear. We examined the effects of contaminants in urban stormwater on macroinvertebrate communities, specifically whether exposure to contaminated sediments alters macroinvertebrate drift behavior and survival. Using a custom built filtration system and experimental stream channels on a stream in Seattle, WA, we exposed diverse macroinvertebrate communities to either filtered ("clean") or unfiltered (ambient) stream water for several three-week experiments. Analysis of chemistry samples indicated there were differences between treatments (e.g. reduction of polycyclic aromatic hydrocarbons and metals in filtered treatments relative to unfiltered treatments), and we observed differences in the drift behavior and survival for some sensitive macroinvertebrate taxa. Such reductions in sensitive invertebrates may help explain why communities in chronically-exposed urban watersheds are depauperate, and why species that rely on invertebrate production, such as endangered salmonids may be at risk as well.

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DETERMINATION OF THE ACUTE TOXICITY OF BENZENE TO *EISENIA FETIDA* IN ARTIFICIAL SOIL OVER 7 DAYS

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The experiment simulated a hydrocarbon spill by exposing different concentrations of benzene to *Eisenia fetida* (red worms). Mortality was the main observation while other observations included pigment, relative size, and amount of movement of the red worms. Three concentrations of benzene were used, 5.0 ml, 10.0 ml, and 20 ml, for the first part of the experiment as a range finding test. The second part had a more narrow range of concentrations to find the LC50 which was 22 g/kg benzene. For seven days the worms lived in artificial environments dosed with benzene, observations were taken and the numbers of living and dead red worms were recorded. The data results showed at higher concentrations of benzene, the number of dead worms found in soil or exhibited avoidance behaviors increased. In summary, increased amounts of benzene resulted in increased numbers of dead worms, but that some of the 'more fit' red worms could survive in the environment at concentrations that others could not.

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THE EFFECT OF PRE-EXPOSURE ON *IN VITRO* BIOTRANSFORMATION RATES OF HYDROPHOBIC CHEMICALS IN RAINBOW TROUT (*ONCORHYNCHUS MYSKISS*)

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There is much effort to develop in vitro methods to measure metabolic transformation rates of chemicals manufactured in high volumes to better assess the potential of chemicals to bioaccumulate in organisms. Knowledge and data of chemical biotransformation rates in fish and other organisms have been identified as key requirements for the environmental evaluation of commercial chemicals. A criticism of *in vitro* methods for measuring metabolic rates is that the animals used as the source of liver metabolizing enzymes may not be representative of organisms in real environments. Aquatic environments in particular are reservoirs for many contaminants and allow for organisms to be exposed to multiple pollutants. Organisms may be exposed to chemicals that inhibit or induce biotransformation enzymes, which can impact the ability of an organism to metabolize chemical substances. This has been studied extensively with industrial and pulp and paper mill effluents, but limited studies have explored the effect of treated municipal wastewater effluents. Using organisms that represent in situ situations may provide more accurate laboratory assessment when measuring chemical metabolic rates in animals for bioaccumulation assessment. The current study used liver S9 homogenates to determine the effect of pre-exposure on in vitro biotransformation rates of polycyclic aromatic hydrocarbons (PAHs) in rainbow trout (*Oncorhynchus mykiss*). Pre-exposure to secondary treated wastewater effluent from a municipal source took place over a 7day period. Fish were exposed under static conditions to varying environmentally relevant dilutions of secondary treated wastewater effluent at 0, 0.1, 1 and 10%. Biotransformation rates of rainbow trout liver s9 samples were measured for each exposure group via the conventional substrate depletion method. The depletion rates of individual PAHs will be compared between exposure groups to see if there is a significant influence of pre-exposure on biotransformation rates.

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THYROID GLAND HISTOPATHOLOGY OF A NATIVE NORTH AMERICAN SPECIES, *PSEUDACRIS REGILLA*, IN THE AMPHIBIAN METAMORPHOSIS ASSAY

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The objective of this research was to determine the effects of thyroid hormone treatment on the Pacific Tree Frog (*Pseudacris regilla*) using an established OECD protocol for an African species *Xenopus laevis*. Tadpoles were exposed to thyroxine in premetamorphosis stage for 21 days and then samples of the thyroid gland were obtained to determine whether atrophy or hypertrophy of the gland had taken place along with other morphometric measures. Samples were taken from each tadpole using a microtome and stained using a histology procedure of hematoxylin and a counter stain 0.1% eosin Y and then matched to controls. A severity grading system was used to score the samples based on core criteria including atrophy/hypertrophy, follicular cell height/shape, and follicular hyperplasia. Each sample is compared to the control and given a grade from 0 (not remarkable) to 3 (severe). Samples were also analyzed using computer software to give an exact measurement of each gland. The data collected concluded that a general trend of atrophy of the thyroid gland was evident in the high thyroid hormone treatments with further analyses to be conducted. These are the first of such results for this particular species and they showed great promise that the OECD protocol is transferable to study other amphibian species. Such research opens the door to study other substances that may disrupt thyroid hormone mediated development.

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ESTIMATING IMPACTS ON COHO SALMON POPULATIONS FROM LAND USE-RELATED SPAWNER MORTALITY IN URBAN STREAMS

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Monitoring efforts evaluating urban stream restoration effectiveness in the greater metropolitan area of Seattle, Washington, have detected high rates of premature mortality among adult coho salmon (*Oncorhynchus kisutch*) in restored spawning habitats. Affected animals display a consistent suite of symptoms (e.g., disorientation, lethargy, loss of equilibrium, gaping, fin splaying) that ultimately progresses to death within a few hours. Annual rates of pre-spawn mortality (PSM) observed over multiple years across several drainages have ranged from ~20% to 90% of the total fall run within a given watershed. The current understanding of coho PSM is that it occurs when pollutants accumulate on impervious surfaces during summer and early fall dry periods are washed into coho-bearing streams by fall storm events. The phenomenon seems to require both specific land uses and precipitation patterns. To evaluate the relationships between PSM, land use and precipitation patterns, we ran a series of spatial analyses to detect correlations between land cover (roadways, impervious surfaces, forests, etc.), seasonal rainfall patterns and PSM rates in 6 watersheds. The relative proportion of local roads, impervious surfaces, and commercial property in the catchment was most strongly correlated with coho PSM rates. Analyses suggest that as urban expansion continues, areas that once supported coho salmon may experience PSM rates that could jeopardize the coho salmon population viability. To evaluate the potential consequences of urbanization on wild coho salmon, we constructed life-history models to estimate the impacts of pre-spawn mortality on coho populations and metapopulations. At the low (20%) and high (90%) ends of the range of observed mortality, model results indicated the mean time to extinction of localized coho populations in 115 and eight years, respectively. The presence of productive source populations (i.e., unaffected by pre-spawn mortality) within a metapopulation reduced local extinction risk. However, as more populations within a metapopulation become affected by spawner die-offs, the source population's productivity declined. These simple models demonstrate the potential for rapid losses from coho populations in urbanizing watersheds.

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THE EFFECT OF ATRAZINE AND ROUNDUP® ON *DAPHNIA MAGNA*

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We are conducting a 21 day chronic exposure of atrazine and Roundup®, two prevalent herbicides in the United States, to *Daphnia magna*. Examining the consequences of herbicide interactions is important for understanding the potential synergistic effects of agricultural runoff mixtures. Five concentrations of atrazine are being tested (.5, 1, 1.25, 1.5, 2mg/L) and the mixture treatments are being conducted with atrazine at those concentrations along with Roundup® (450 mg/L, 2% glyphosate) in the U.S. Effects on behavior and reproduction are being monitored several times a week with reconstitution of the treatment solutions once every week. From this experiment we will be able to conclude chronic effect of Roundup® and atrazine concentration separately as well as the combined mixture. We anticipate that there will be decreasing reproduction rates with increasing concentrations of atrazine and further inhibition with the mixture treatments. Our results and its implications will be available at April's SETAC meeting.

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APPLICATIONS OF GEOGRAPHIC INFORMATION SYSTEMS TO ECOLOGICAL RISK ASSESSMENT AND MODELING IN THE SOUTH RIVER AND UPPER SHENANDOAH RIVER, VIRGINIA

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The empowering functions of Geographic Information System (GIS) in gathering, analyzing, querying and displaying spatial data provide an effective platform for generating ecological risk assessments and environmental decision support systems. To demonstrate this, GIS was used to facilitate the creation of a cumulative integrated risk assessment for heavily stressed portions of the South River near Waynesboro, Virginia. Specifically, we used multiple geospatial analyses to construct Bayesian network models and relative risk models (RRM) that evaluate the potential impacts of mercury and other stressors to fish, wildlife, and other stakeholder derived endpoints in the South River watersheds. All models were parameterized using spatial data from a variety of sources including the U.S. Geological Survey, Virginia Department of Environmental Quality, and the South River Science Team. This work highlights the utility of GIS in all stages of the project, from beginning steps (designation of risk regions, data mining) to creation of ranking systems, to final risk calculations and quantitative risk mapping and graphics production. With geospatial information projected at multiple scales, the application of GIS is shown to ultimately aid in the illustration of results of different types of risk analyses, the identification of data gaps, and the prioritization of additional investigations and remedial efforts.

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O_2, CO_2, AND PRESSURE IN AQUATIC CLOSED ECOLOGICAL SYSTEMS

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The assumption that Aquatic Closed Ecological Systems will remain at approximately atmospheric pressure is incorrect if primary productivity is high, such as when nutrient rich solutions and $NaHCO_3$ as the carbon source are used. The O_2 produced will distribute between the head-gas and liquid, in accordance with Henry's Law, and total pressure will increase. This had been published earlier, in studies in which pressure changes were used as a surrogate for O_2 changes. At 20°C, 29.9 times as much O_2 will be in the gas phase than in the liquid phase, per unit volume. The smaller the proportion of head-gas volume, the higher the pressure produced. In conditions measured here, 250 to 1000 ml CESs, mixing is necessary for the pressure and O_2 measurements to agree; in the absence of mixing in these systems, pressure increases lag during the lighted period and persist into the dark period; dark respiration would be seriously underestimated. Given that high pressures can develop (> 760 mm Hg above atmospheric), it is necessary that seals be tested under maximum likely pressures. In some earlier studies in our laboratory, leakage was discounted because tests were run under lesser pressure. In some of these earlier experiments, much less O_2 appeared to be released than carbon fixed. With tight seals, simultaneous measurement of pressure, O_2, pH, and temperature, and by considering both the gaseous and dissolved O_2, diurnal O_2 and CO_2 changes agree quite closely. By considering the O_2 produced and the relative head-gas and liquid proportions, pressure increase and potential leakage can be minimized.

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CONTAMINATION AND TOXICITY OF SNOWPACK COLLECTED FROM SNOWMOBILE RECREATION AREAS

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There is conflicting research as to the amount of unburned fuel and other pollutants emitted by snowmobiles, particularly two-stroke snowmobiles that comprise the bulk of snowmobiles in use. Snowpack samples were collected from snowmobile recreation areas in Mt. Baker-Snoqualmie National Forest, Washington and the Bridger-Teton National Forest, Wyoming in February and March, 2011. Samples were from snowmobile trails and nearby locations that were thought to have negligible snowmobile use. Chemical analysis of the samples is currently being conducted for analytes that include BTEX, heavy metals, and major anions and cations. The Microtox acute toxicity test is being conducted on each of the samples. These analyses are on-going, but the results are expected to contribute an understanding to the potential impacts of snowmobile usage.

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