Welcome to the CLU-IN Internet Seminar

Innovative Technologies Used to Quantify Environmental Contaminant Bioavailability and Characterize Environmental Exposure National Institute of Environmental Health Sciences, Superfund Research Program

Sponsored by: U.S. EPA Technology Innovation Program

Delivered: January 30, 2012, 2:00 PM - 4:00 PM, EST (19:00-21:00 GMT)

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Although I'm sure that some of you have these rules memorized from previous CLU-IN events, let's run through them quickly for our new participants.

Please mute your phone lines during the seminar to minimize disruption and background noise. If you do not have a mute button, press *6 to mute #6 to unmute your lines at anytime. Also, please do NOT put this call on hold as this may bring delightful, but unwanted background music over the lines and interupt the seminar.

You should note that throughout the seminar, we will ask for your feedback. You do not need to wait for Q&A breaks to ask questions or provide comments. To submit comments/questions and report technical problems, please use the ? Icon at the top of your screen. You can move forward/backward in the slides by using the single arrow buttons (left moves back 1 slide, right moves advances 1 slide). The double arrowed buttons will take you to 1st and last slides respectively. You may also advance to any slide using the numbered links that appear on the left side of your screen. The button with a house icon will take you back to main seminar page which displays our agenda, speaker information, links to the slides and additional resources. Lastly, the button with a computer disc can be used to download and save today's presentation materials.

With that, please move to slide 3.

INNOVATIVE TECHNOLOGIES USED TO QUANTIFY ENVIRONMENTAL CONTAMINANT BIOAVAILABILITY AND CHARACTERIZE ENVIRONMENTAL EXPOSURE

Kim A. Anderson, PhD
Professor, Environmental & Molecular Toxicology
Director, Food Safety & Environmental Stewardship Program
Oregon State University

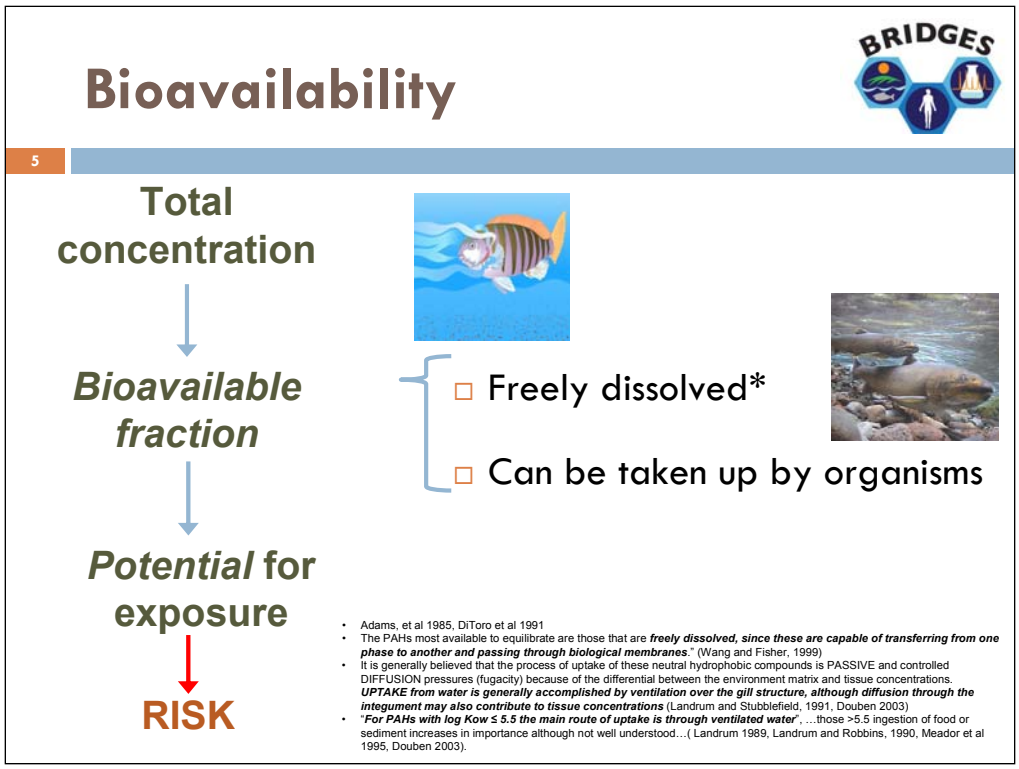
Why Bioavailable ?

Environmental exposure and fate

- *Understanding environmental factors on diseases…*
  - Must develop new bio-analytical tools to measure exposure
    - L.S. Birnbaum, EHP, 2010

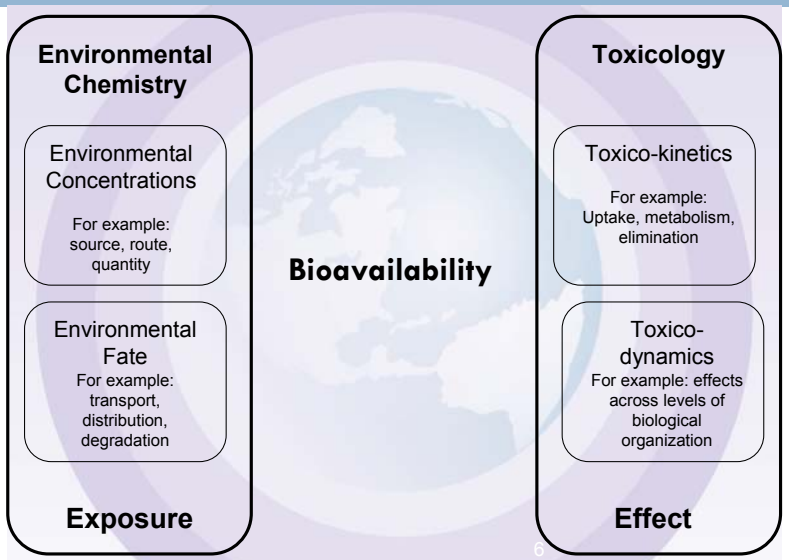
Thinking outside the sampling jar

- Intelligent sampling
- Environmental exposure
- Bioavailability

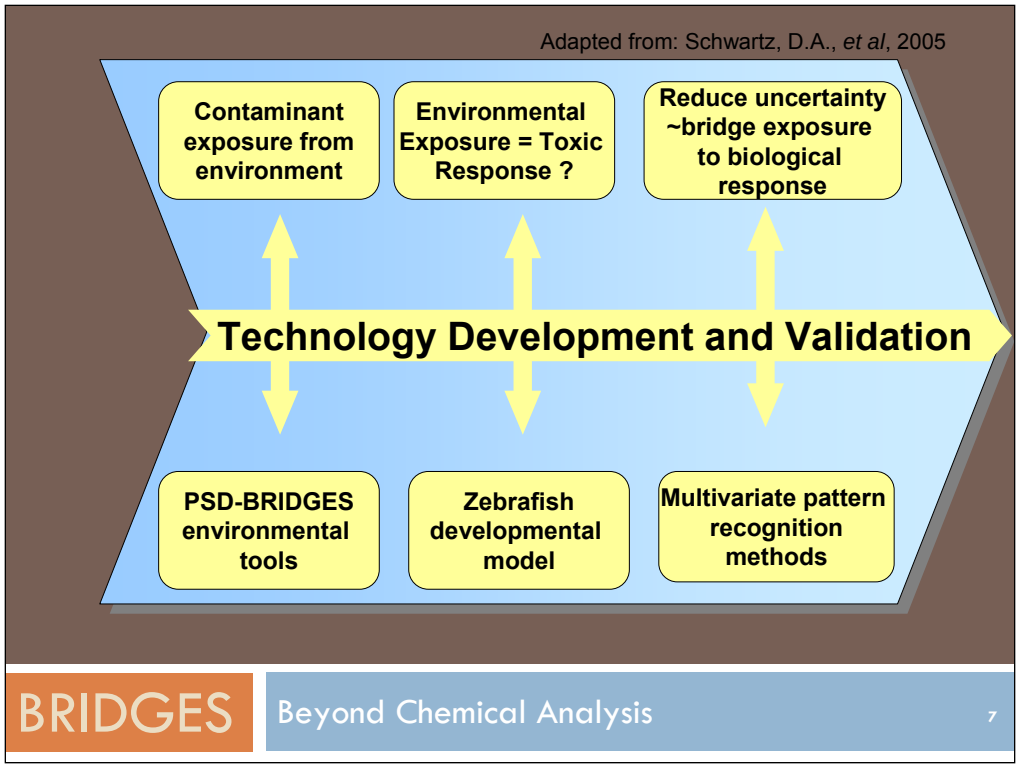


The presence of toxic chemicals in the environment is not necessarily indicative of a risk to human and environmental health; the chemicals must be bioavailable in order for there to be an exposure, which is a necessary precursor to a toxic outcome. The bioavailable fraction of a chemical is the portion of the total bulk concentration that is capable of being taken up by organisms and is sometimes referred to as the external dose *(1)*. Bioavailability is therefore a more biologically relevant measure of contamination and potential exposure than total concentration.

BRIDGES



Adapted from: Anderson & Hillwalker, Ecotoxicology Bioavailability, Elsevier 2008


Adapted from: Schwartz, D.A., *et al*, 2005
Contaminant exposure from environment
Environmental Exposure = Toxic Response ?
Reduce uncertainty ~bridge exposure to biological response
Technology Development and Validation
PSD-BRIDGES environmental tools
Zebrafish developmental model
Multivariate pattern recognition methods
BRIDGES
Beyond Chemical Analysis
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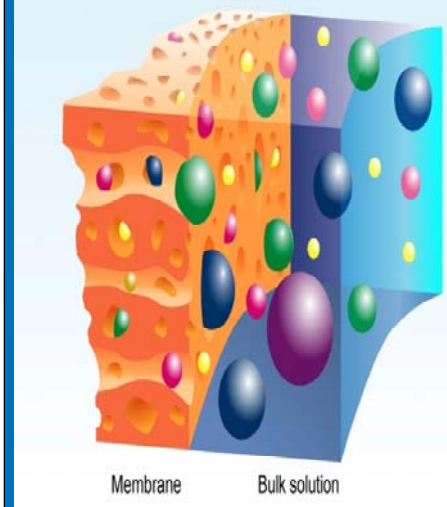
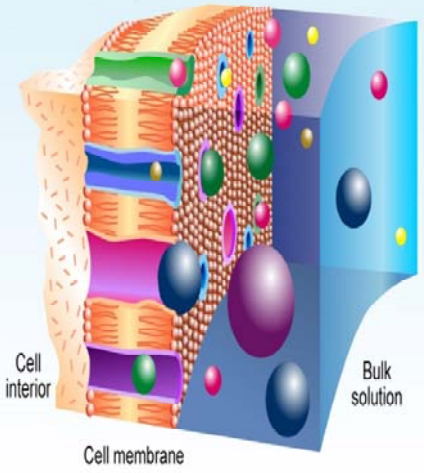
PSD Membrane



Cell Membrane



Passive sampling devices *quantitative* technology for deployment at Superfund sites

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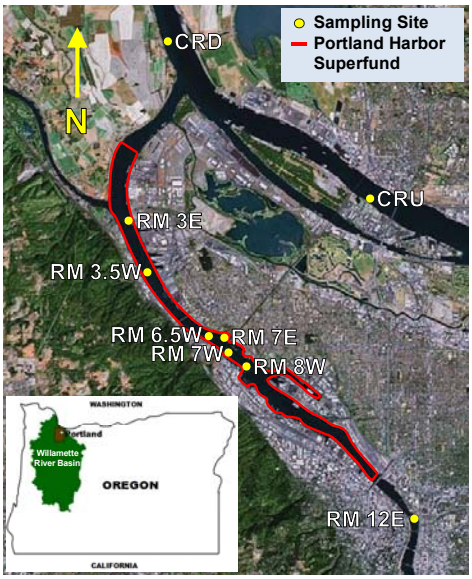
- **PSD theory:** PSD represent an organic lipid membrane. Like a membrane, PSDs discriminate against particulate bound material. As *in situ* time integrative passive samplers, PSDs may be deployed for extended periods of time to sequester contaminants. This **overcomes potential issues such as detection limits, bioavailable fraction collection and fluctuating contaminant concentrations.** ***In-situ*****, site specific calibration is through isotope labeled infusion into the PSD with performance reference compounds.**

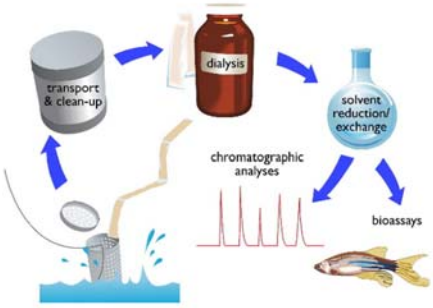
Anderson, K.A.*, Sethajintanin, D, Sower, G. and Quarles, L. "Field trial and modeling of uptake rates of in situ lipid free polyethylene membrane passive sampler" *Environ. Sci Tech.* 2008.

Contributions to Understanding Environmental Exposure

Goal: Protection of Human Health



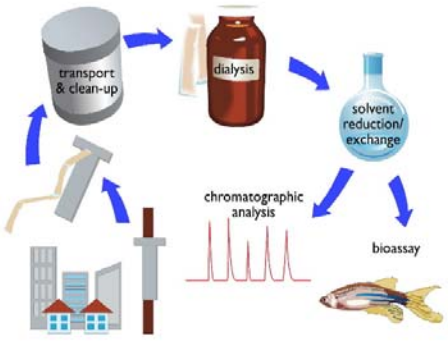
Water Passive Sampling



Anderson, et al; ES&T, 2008

Bioavailable Passive Samplers
Holistic: Water, Air, Sediment, and Surrogate

Extraction and bioassays



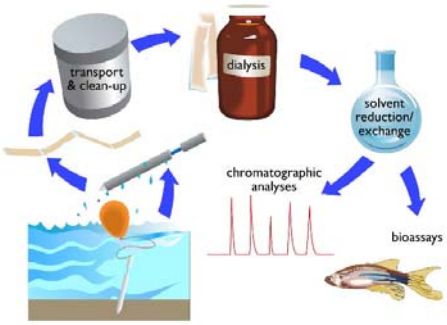
Common Metric of Exposure

PSD: Relevant

Numerous Chemistry Opportunities

- Extract clean
- Pesticides, PCBs,
- 1,200+ analyte screen
- LC or GC compatible
- PAHs (EPA) and beyond33 PAHs
- 302 mw, dibenzopyrene isomers PAHs
  - Layshock *et al* JEM, 2010
- Oxygenated PAHs (ketones, quinones)
  - Layshock and Anderson, ETC, 2010

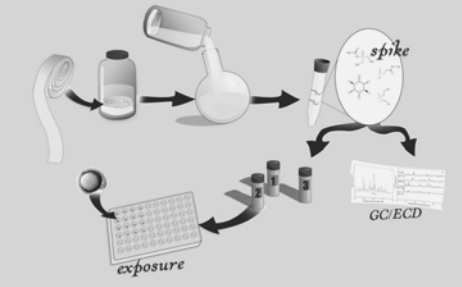
Numerous Bioassay Opportunities (in vivo and in vitro)



Preparation and Method of Extraction

Hazard reduction, labor reduction, solvent reduction, recycling solvents

- Pre-clean LDPE w/ hexane
  - Also available from OSU SRP
- Infuse /add PRC
  - Performance Ref Cmpds- perdeuterated
- Post deployment
  - HCl /isoproponal removes debris & water



Anderson, et al; ES&T, 2008

- Storage stability study, sealed cans until used 6 mo
  - Recoveries for PRCs ± 10% of TV
- Transportation stability tested in Teflon™ at 50C for 2 weeks
  - Recoveries from storage stability ± 10 True Value (TV)
- Transported in Teflon™ bags
  - Teflon bags extracted to check
  - Teflon extracts BDL for all target analytes

Method of Extraction and Analysis

Hazard reduction, labor reduction, solvent reduction, recycling solvents

- PSD extracted w/ 40 mL of hexanes for 4 hrs, let stand, repeated once for 2hrs.
  - An 80% reduction compared w/ SPMDs
  - Eliminates all methylene chloride compared with ~100 mLs per ea SPMD
- Concentrated to 1mL
  - Eliminates 2 solvent reduction steps compared with SPMDs
- All solvents collected for recycling
  - TurboVap™ 500 Closed Cell evaporator (or equivalent)
  - TurboVap™, Zymark LV evaporator (or equivalent)

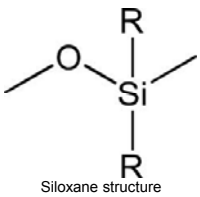
Anderson, et al; ES&T, 2008

- Agilent 5975B Gas Chromatograph-Mass Spectrometer (GC-MS);
  - DB-5MS column (30 m x 0.25 mm x 0.25 µm) in electron impact mode (70 eV) using selective ion monitoring (SIM). The GC parameters were as follows: injection port maintained at 300 °C, 1.0 mL min^{-1} helium flow, 70 °C initial temperature, 1 min hold, 10 °C min^{-1} ramp to 300 °C, 4 min hold, 10 °C min^{-1} ramp to 310 °C, 4 min hold. The MS temperatures were operated at 150, 230 and 280 °C for the quadrupole, source and transfer line respectively.

Complementary PSD also include silicone PSD

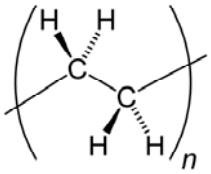
- Anderson Lab:
  - PCB congners (LFT)
  - Pesticides (LFT log K_{ow} >3)
  - PAHs (LFT)
  - PBDEs (LFT in prog)
  - **Metals (DGT)**
  - **Oxy-PAHs (Si)**
  - **Pesticides (Si log K_{ow} <5)**

Silicone



Siloxane structure

LFT



Polyethylene structure

Ha: If inherent intermolecular forces in PSDs drive adsorption for target compounds, then PSDs of different materials should preferentially target different compounds

Passive Sampler Comparisons (PAHs, PCBs, …)

NDA=No Data available
Mixed= some literature indicating no, NA = not applicable

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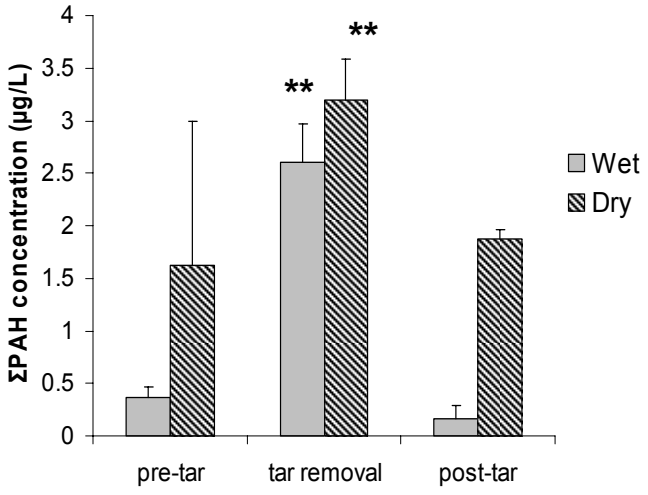
Passive Devices *our lab	Possibility of time weighted averages	Suitable for long & short term collection	Compatible w/ in vivo and in vitro	Eliminate need for Cl-solvent	Field In situ Calibration	Eliminate further cleanup (GPC, soxhlet)	Extract available for future analyses	Suitable for turbulent waters
LFT*	YES	YES	YES	YES	YES	YES	YES	YES
Si*	YES	YES	Inprog	YES	YES	YES	YES	YES
SPMD	YES	YES	possible	NO	YES	NO	YES	YES
POM	Mixed	Mixed	NDA	YES	NDA	Mixed	YES	YES
SPME	NDA	NO	YES	YES	NDA	YES	NO	NO
EVA	YES	YES	NDA	NO	YES	NO	YES	NDA
Tenax	Mixed	NO	NO	YES	Mixed	YES	NO	NA
POCIS	Mixed	YES	Mixed	Mixed	NO	YES	YES	NO

LFT= lipid free tube (LDPE), Si=silicone (polysiloxane), SPMD=semipermeable membrane device, polydimethylsiloxane (PDMS), polyoxymethylene (POM), polyacrylate (PA= SPME), ethyl vinyl acetate (EVA). PMDS= Silicone (R=dimethyl).

Superfund Remediation: Effect of dredging at RM 6.3 on *bioavailable* PAHs at Portland Harbor, RM 7

Increase during remediation, residuals following, reduction later

n=3, each a composite of 5, $p<0.05$, in situ calibration with labeled PRC



Sower & Anderson, ES&T, 2008

Contaminants Vary BOTH Spatially and Temporally at Superfund sites

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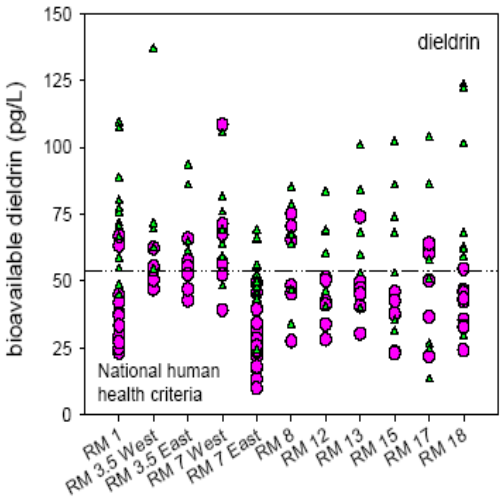
- Water quality data for the carcinogenic EPA PP PAHs.
- ▲/○ = wet season
- ●/● = dry season
- The dashed lines represent the EPA Water Quality Guidelines for human health for consumption of water and organism.



N = 110, P<0.001, 3 week deployments
● = low flow (summer)
▲ = high river flow (>10,000 ft^3/s, >1 in rain)

Sethajintanin and Anderson, ES&T, 2006

Contaminants Vary BOTH Spatially and Temporally at Superfund sites

19

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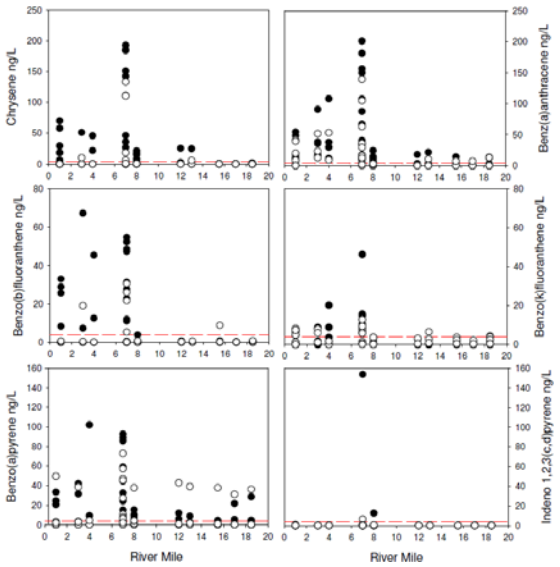

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Sower & Anderson, ES&T, 2008

BRIDGES: **Reduce exposure uncertainty by analyzing biological responses**

BRIDGES extracts with bioassay model (Zebrafish, Ames, etc) system

BRIDGES well suited for effects-directed analysis

BRIDGES designed for relevant mixtures

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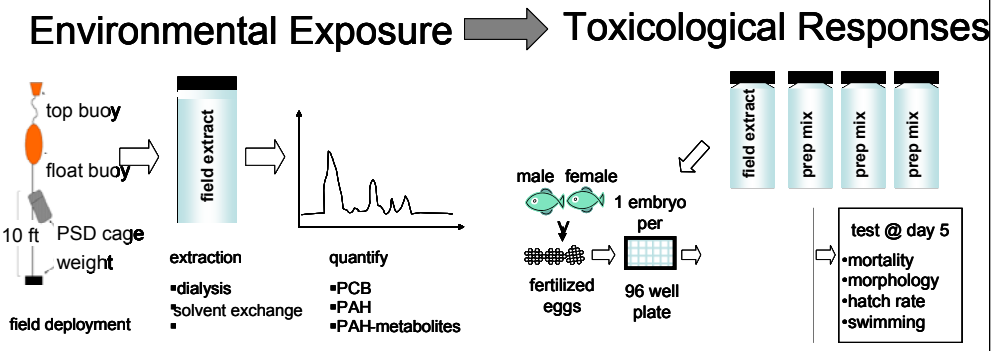


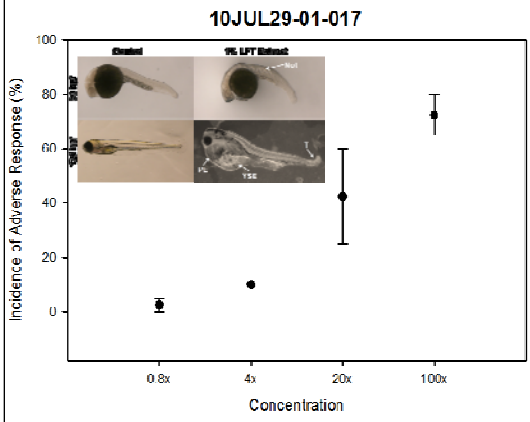
Hillwalker, Allan, Tanguay, & Anderson Chemo. 2010

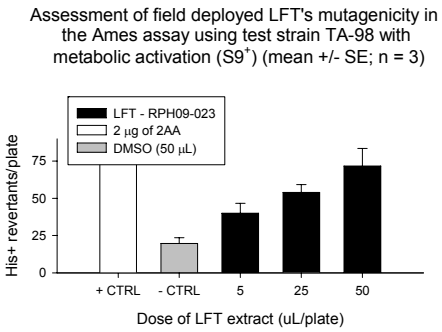
PSD Integrated with Two Bioassays

in vivo and *in vitro* embryonic zebrafish model, Ames test

PH Superfund RM =3.5W

PH Superfund RM = 7W





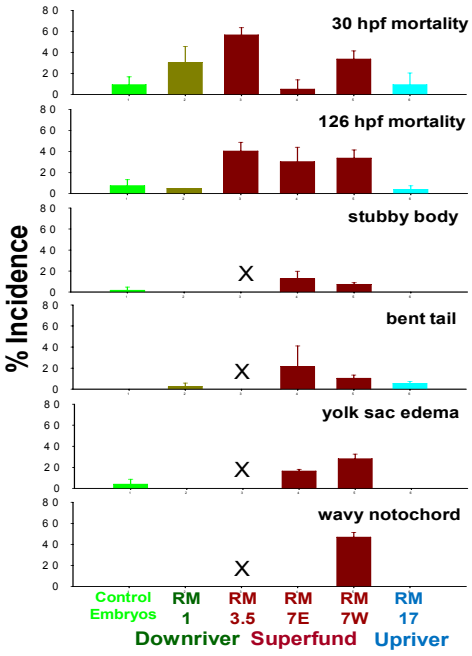
Site-specific Biological Responses

BRIDGES

6 of 18 biological responses were significantly different in exposed embryos compared to controls

MLR, likelihood ratio, $p<0.05$;

n = 941



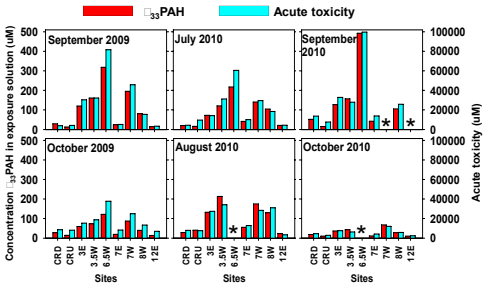
Hillwalker, Allan, Tanguay, & Anderson Chemo. 2010

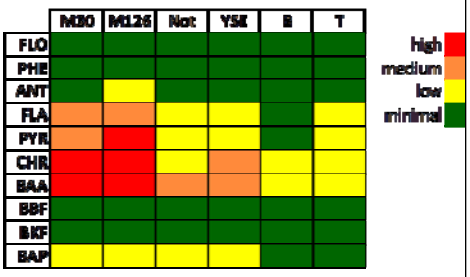
Mixtures: Discriminatory pattern recognition and multivariate regression assessments of components in PSD extracts and signatory biological responses

Acute toxicity:

Log LC_{50} (mM/L) = -1.162 log K_{ow} + 2.496

"Heat diagram" Matrix Bio-Chem





Estimating exposure (risk) at a Superfund site using PSDs as biological surrogates in human health risk models

- Apply PSD data in a Public Health Framework
  - PSDs may be used as a biological surrogate
  - Added spatial and temporal variations in potential human health estimate of exposures
- Method Calculating Exposure
  - Mass-to-mass concentrations of PAHs in PSDs were substituted for fish tissue concentrations

- Exposure from consumption of resident organisms
  - Tissue contaminant data
    - Difficult to obtain fish/shellfish
    - Destructive sampling
    - Inherent biological and physiological variability
    - Limited spatial/temporal information

Allan, Sower & Anderson, Chemo. 2011

Comparison of PSDs and fish tissue

Sampling periods overlapped, however, PSD from this study, fish data from Lower Willamette Group Harbor RI/FS except where noted or Data from Portland Harbor Public Health Assessment. NA indicated not available. Allan, Sower & Anderson, Chemo. 2011

FISH data from: Integral, Windward, Kennedy/Jenks and Anchor-QEA PH RI/FS Remedial Investigation Repot, IC09-0003, 2009. Prepared for the LWG Portland and ATSDR, U.D.o.H.a.H.S., Public Health Assessment and EPA Facilty IC OR0001297969, 2006

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	Concentration (ug/kg) - Average (Maximum)						
	PSD		Fish and Shellfish from Superfund				
Chemical	Superfund	Upstream	Smallmouth Bass	Carp	Sculpin	Crayfish	Clam
Naphthalene	1.0 (6.5)	0.7 (3.8)	10 (86)	20 (56)	19 (250)	0.82 (2.9)	25 (78)
Acenaphthene	5.5 (54)	0.02 (1.1)	13.7 (95)	34.1 (75)	NA	NA	NA
Fluorene	13 (84)	5.4 (70)	9.31 (69)	22.3 (53)	NA	NA	NA
Phenanthrene	44 (219)	4.9 (24)	20 (85)	10 (16)	6.8 (33)	52 (97)	35 (300)
Fluoranthene	170 (850)	24 (57)	2.77 (36)	NA	NA	10.2 (130)	NA
B(a)anthracene	51 (504)	10 (44.6)	NA	NA	NA	2.01 (80)	NA
Chrysene	36 (172)	10 (28)	20 (85)	NA	NA	2.16 (87)	NA
Pyrene	170 (733)	35 (92)	2.9 (39)	NA	NA	4.02 (83)	NA
B(a)pyrene	14 (70)	4.1 (21)	0.64 (1.3)	NA	NA	1.1 (7.5)	34 (490)
Σ_{16}PAH	819 (3094)	397 (1147)	71.5 (308)	85.5 (222)	52.3 (550)	71.2 (477)	478 (4980)
Σc PAH	23 (123)	7.6 (25.2)	2.5 (6.8)	2.1 (2.8)	3.18 (9.8)	22 (170)	220 (2700)

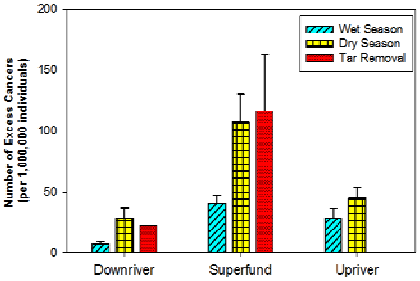
comparisons of PAH concentrations in PSDs and fish tissue from the Portland Harbor Superfund site demonstrate that using PSD concentrations in a public health assessment would provide a reasonable and conservative estimate of exposure that would be protective of human health without significantly overestimating risk. Table 3.1 presents fish tissue data from the Lower Willamette Group *(21)*, some of which was used in the Portland Harbor Public Health Assessment *(16)* as well as PSD data from this study. The fish and shellfish were collected from Portland Harbor during a period that overlapped with the PSD study; however these two studies are unrelated to one another. Furthermore, it is important to highlight that PAHs were not included in the Portland Harbor Public Health assessment because of insufficient data *(16)*; therefore, the data presented in Table 3.1 is based on a limited sample set. The side-by-side comparison demonstrates that PSDs from this study captured the magnitude, range and variability of PAH concentrations that have been reported in a variety of fish and shellfish tissues from the harbor and provide an estimate of exposure that is realistic and protective.

Estimating Exposure = $\frac{C \times IR \times EF \times ED}{BW \times AT}$

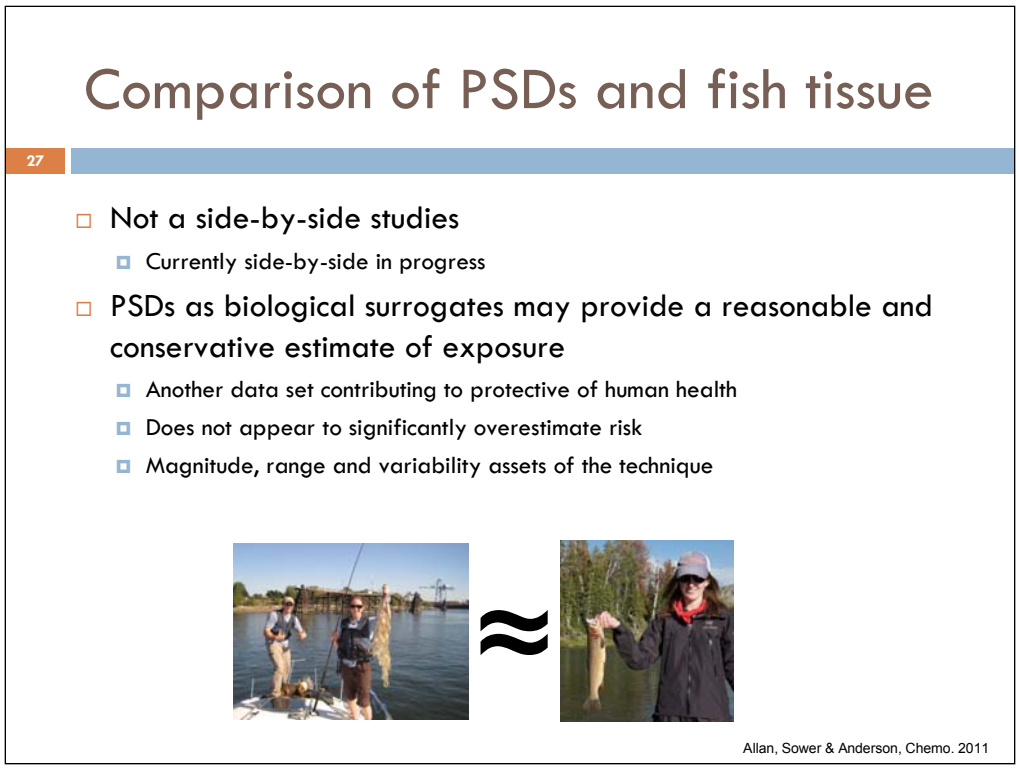
Excess LIFETIME Cancer Risk = Exposure x Slope Factor

Exposure Estimation Variables			
Parameter		Adult	Units
Concentration	C		ug/g
Ingestion Rate Average	IR-AVG	17.5	g/day
Ingestion Rate High	IR-high	142.4	g/day
Body Weight	BW	70	kg
Exposure Frequency	EF	365	days/year
Exposure Duration	ED	30	years
Averaging Time	AT-non-cancer	10950 (30)	days (years)
Averaging Time	AT-cancer	25550 (70)	Days (years)

Cancer risk based on average consumption



UNCERTAINY: PSD-fish, PSD do not move, do not metabolize or excrete, passive uptake not dietary, may overestimate for finfish, underestimate shellfish, PSD have some of the same assumptions as fish data such as, consumption rates, consumption patterns, fish preparation, PSD and fish site and time specific, below detection limits ……..



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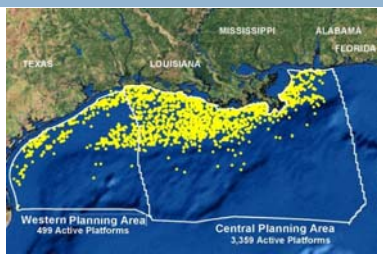
Gulf of Mexico: Goals many fold
Many ideally suited to passive samplers

- PRE-spill conditions
- Establish regional and *individual* relationships
- Oil trajectory uncertain
- Broad geographic areas "at risk"
- **Defensible, Unbiased data**
  - Passive Sampler for **aquatic exposures and** PSD **air** sampler
  - Suitable for chemical mixtures
  - Both **chemical and bio-assays**
  - Quality Control, PRC



Grand Isle, LA, Research June 2010 Sampling Campaign (photo: KA Anderson)

Gulf Port, MS

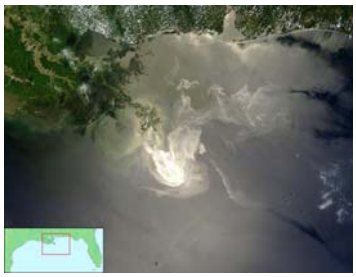
Bon Secour, AL

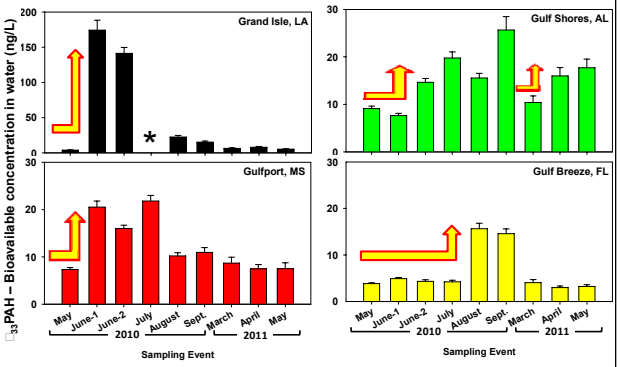
Grand Isle, LA

Gulf Shores, FL

Temporal, Spatial PAHs Gulf of Mexico

Bioavailable Water Concentrations of PAHs (ng/L)





Allan, Smith & Anderson, ES&T 2012, in press

Analyzing the chemical 'fingerprint' in a congener profile (petrogenic – pyrogenic)

PAH ASEMBLAGE SERIES

1. Parent,

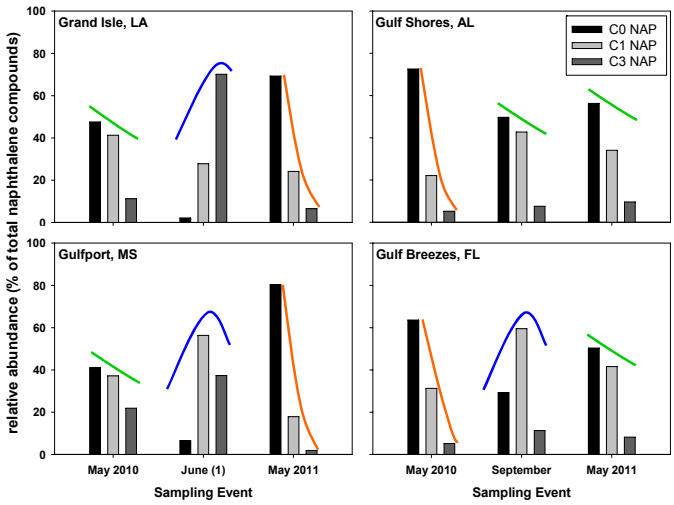
2. 1-Alkyl-PAH

3. 3-Alkyl-PAH

Mixed

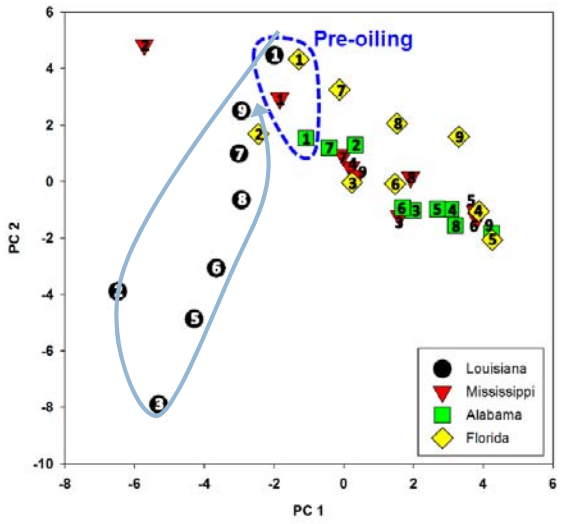
Petrogenic

Pyrogenic



Principle Component Analysis

#1- #9 = May 2010 - June 2011



Allan, Smith & Anderson, ES&T **2012**, in press

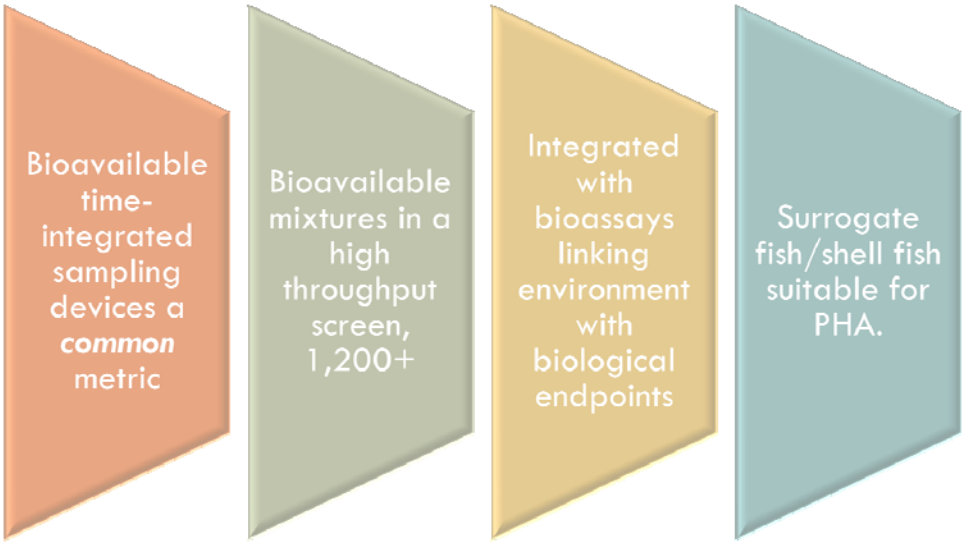
Estimating Exposure and Bioaccumulation using PSDs*

PSD Range of $\sum PAH_{33}$ ug/Kg	US FDA ** Total Mean PAH ug/kg	US FDA ** Total Mean PAH ug/kg	US FDA ** Total Mean PAH ug/kg	US FDA ** Total Mean PAH ug/kg
PSD	Oyster tissue	Crab	Shrimp	Finfish
68 - 6,000	3,676	411	56.9	21 - 143

PAH conc in PSDs in this study comparable to salmon and mussel tissues conc observed in the Gulf of Alaska following the Exxon Valdez sill.***

*Allan, S. E.; Sower, G. J.; Anderson, K. A *Chemosphere* **2011,** *85*, (6), 920-927.
Gohlke, J. M.; Doke, D.; Tipre, M.; Leader, M.; Fitzgerald, T., A t. *Environmental Health Perspectives* **2011, *119*, (8).
***Neff, J. M.; Burns, W. A., E*nvironmental Toxicology and Chemistry* **1996,** *15*, (12), 2240-2253.
*Allan, S.E. Smith B.W. & Anderson, K.A. Environmental Science and Technology, **2012**, in press

Anticipated IMPACT



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http://fses.oregonstate.edu

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http://oregonstate.edu/superfund/oilspill

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PSD used in Africa:
Beautifully suited to challenges

Chemical Reaction Kinetics Model
uptake and release of contaminant

Air/Water | Passive Sampling Device | Air/water

Rate to change of the concentration:

$$dC_m/dt = k_u C_w - k_e C_s$$

38 Conc at any *t* is determined by competing rates of uptake and release

Anderson, et al; ES&T, 2008

Water concentrations were calculated using the empirical uptake model with PRC-derived sampling rates, which is explained in detail in Huckins et al. (2006) [1]. Sampling rates (R_s) for performance/permeability reference compounds (PRC) are estimated from (Eq. 1)

$$R_S = -\frac{\ln\left(\frac{N}{N_0}\right)}{t} K_{SW} V_S$$

where N and N_0 are the PRC amounts at the end and beginning of the exposure respectively, K_{SW} is the sampler-water partition coefficient, V_S is the sampler volume and t is the exposure time. If the recovery of a PRC was below 20% or above 80% then the sampling rate was determined using the nonlinear least-squares method detailed in Booij and Smedes (2010) [2] (Eq. 2)

$$f = \exp\left(\frac{R_S t}{K_{SW} V_S}\right)$$

where f=N/N_0 and is a continuous function of K_{SW} and R_S is an adjustable parameter. K_{SW} is calculated for each PRC and target analyte using a regression, based on octanol-water partitioning coefficients (K_{OW}), that was determined for low-density polyethylene (LDPE) samplers [3]

$$\log K_{SW} = 1.05 \log K_{OW} - 0.59$$

The compound specific effect (α) on sampling rates is a function K_{OW} and is calculated for each PRC and target analyte based on the equation [1] (Eq. 4)

$$\log \alpha = 0.0130 \log K_{OW}^3 - 0.3173 \log K_{OW}^2 + 2.244 \log K_{OW}$$

The sampling rates of target analytes for a specific exposure are calculated based on the R_S of the PRC (Eq. 1 or Eq. 2) with the most similar K_{OW} and the ratio between the compound specific effects (Eq. 4) for the PRC and the target analyte [1] using

$$R_{S,target\ analyte} = R_{S.PRC} \times \frac{\alpha_{analyte}}{\alpha_{PRC}}$$

Finally, the water concentration (C_W) of each analyte is calculated based on the amount of the analyte that was sequestered by the sampler ($N_{analyte}$) and the other calculated and measured parameters [1] using

$$C_W = \frac{N_{analyte}}{V_S K_{SW}\left(1 - exp\left(-\frac{R_S t}{V_S K_{SW}}\right)\right)}$$

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