Screening of SF Bay harbor seals to identify CECs

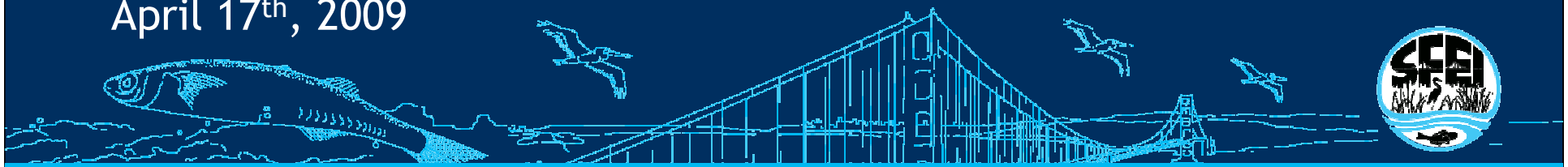
A two-year study proposal for 2010 and 2011

John Kucklick, National Institute of Standards and Technology (NIST)

Susan Klosterhaus, SFEI

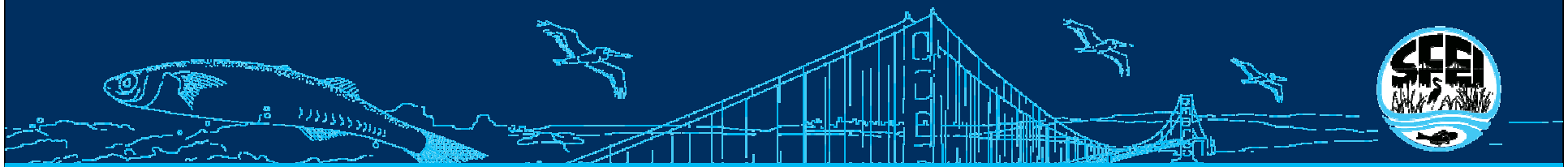
Denise Greig, The Marine Mammal Center

RMP Emerging Contaminants Workgroup Meeting
April 17th, 2009



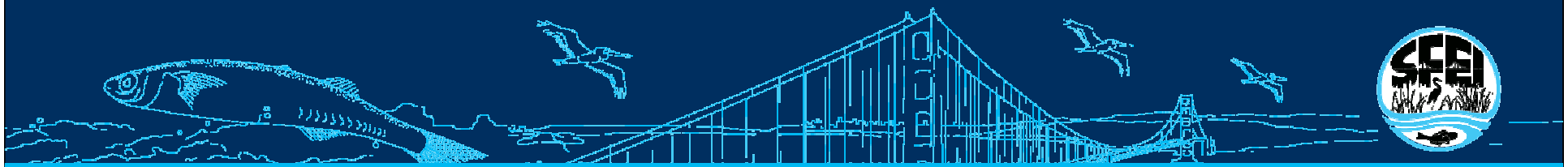
Traditional approach to monitoring:

- Target specific chemicals based on what we know
  - use, toxicity, methods, results from other studies
  - SF Bay: PCBs, OC pesticides, PAHs, PBDEs, PFCs, etc
- Sometimes we find what we are not looking for
  - PBDEs in European samples
  - tetrabromophthalate in PBDE analysis of dust
- **So what about the other 1,000s+++ chemicals in use and their degradation products?**



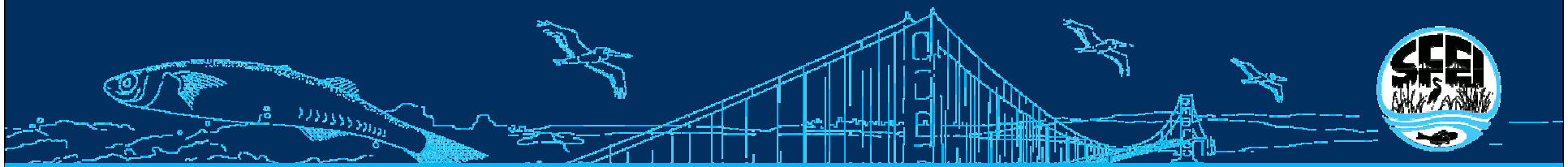
Alternative approach to monitoring

- Screen samples for hundreds (thousands?) of chemicals
- Minimal clean-up, full scan of extracts
- Take advantage of recent advancements in methods and instrumentation (e.g. GCxGC/TOF-MS)
- NIST using similar approach to screen human blood
- More efficient method?



Study Objective

- Identify previously unmonitored, anthropogenic chemicals that may be adversely affecting the Bay foodweb

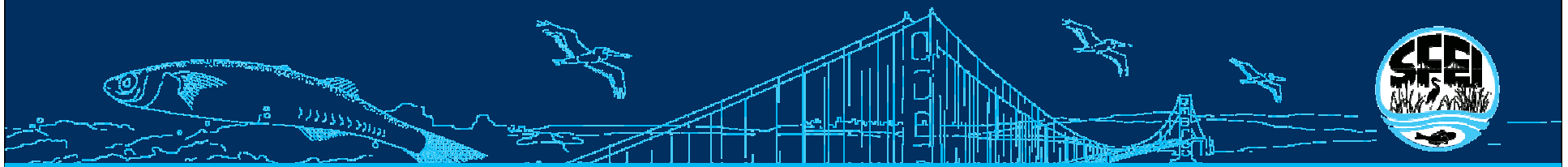


Approach

- Analyze harbor seal blood, blubber, liver -- ideally from same animal

  -- stranded seals, seals euthanized at TMMC, and/or live captures

  -- target ~20 seals
- Analyze seals from Tomales Bay (reference site) if possible



NIST validated methods

1. Legacy compounds
- PCBs, chlordanes, DDT and metabolites, toxaphene congeners, mirex, chlorobenzenes

2. Brominated flame retardants
- Focus on compounds not previously targeted

Hexabromobenzene (HBB)
Pentabromotoluene (PBT)
Dechlorane Plus
BTBPE
PBEB
DBDPE
TBECH (α/β and γ/δ)

2,3,5,6 tetrabromo-p-xylene
allyl 2,4,6 tribromophenyl ether
octabromotrimethylphenylindane
2,3 dibromopropyl 2,4,6 tribromophenyl ether
2-bromoallyl 2,4,6-tribromophenly ether
α, β 1,2,5,6 tetrabromocyclooctane

NIST validated methods

3. Perfluorinated compounds (PFCs)
- Optional since have seal data
- Rely on QTOF for these?
- If possible, samples will be screened for additional PFCs by fluorine NMR

4. Phenolic compounds
- hydroxylated metabolites of PCBs and PBDEs
- derivatization with diazomethane and with extracts analyzed by GC/MS

5. Methyl sulfone compounds
- metabolites of PCBs and DDE
- Detected in arctic mammals
- Non-polar extracts analyzed by GC/MS

6. Trace elements and selected trace element species
- Alkyl tins, selenium (?) determined by LC or GC coupled to ICP/MS

Time of flight (TOF) methods

1. GCxGC TOF (EI spectra)

- Both nonpolar and polar compounds
  -- pharmaceutical metabolites, musks, cyclic/linear siloxanes?
- LECO Peagasus (NIST Gaithersburg)
- Derivatized and underivatized extracts of blood and/or liver
- Underivatized blubber extracts
- Samples will be initially screened against the NIST EI data base (~200k spectra)
- If needed, will attempt to identify unknowns by mass spectral elucidation
- Guidance: Muir and Howard Great Lakes report; Brown and Wania (2008)
  -- Get standards for these compounds (?) and add them to a database
  -- Consult with Steve Stein (NIST) who developed the NIST EI database
- Run calibration mixtures for compounds analyzed using NIST-validated methods along side to help identify these common compounds (quantification optional)
- Used successfully by NIST to screen human blood samples

Time of flight (TOF) methods

2. GC TOF NCI (15m DB-5ms type column)

- Primarily nonpolar compounds
- Waters GC-T (NIST Charleston)
- Extracts of blubber and blood with minimal clean-up
- No NCI database; focus on Br compounds not previously analyzed
  -- e.g. bromocyclohexanes, bromobenzenes, mixed bromo and chloro cyclohexanes
- Guidance: Muir and Howard Great Lakes report; Brown and Wania (2008)
  -- Get standards for these compounds (?) and set retention times
- Run calibration mixtures for compounds analyzed using NIST-validated methods along side to help identify these common compounds (quantification optional)
- Derivatize to look for halogenated polar compounds (e.g. N-Br?)

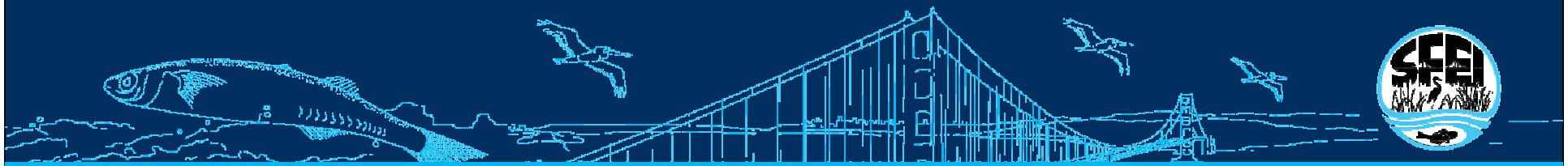
Time of flight (TOF) methods

3. LC-Q-TOF

- Polar compounds less amenable to GC; compliment GCxGC-TOF derivatized sample analysis
- Used successfully in metabolomic work and by USGS for screening water samples near WWTPs
- Waters QTOF Premier with the Aquity nano LC (NIST Gaithersburg)
- Screen extracts of underivatized blood and/or liver
- NIST database contains only a few thousand compounds
- If possible, obtain likely target compounds identified from screening exercises and include these in a library (NIST QTOF data base)
- Other database available from Waters?
- Most exploratory screening method, requiring most work, potential year 2 activity

Analytical Considerations

- Method development required, labor intensive
- Could analyze only a sub-set of samples for lower priority analyses
- Analyses are ‘scalable’ and could be influenced by our priorities



Proposed Budget

	2010 Estimated Costs	2011 Estimated Costs
Sampling supplies	$2,500	$2,500
Analytical standards	$5,000	$5,000
Other laboratory supplies	$2,500	$2,500
Analytical costs, data management, analysis, and reporting	$40,000	$40,000
SFEI project management	$5,000	$5,000
SFEI data management		$7,000
SFEI reporting		$8,000
Total	$55,000	$70,000

How many samples can we collect?

1. Stranded, live animals that are euthanized at TMMC
- High quality samples
- Blood (50 ml), blubber (50g), and liver (50g) from the same animal
- 2 pups and 2 adults from the Bay in 2007/2008
- Include seals from coast, just north and south of the Golden Gate? - would add 1-2 more animals, thought to be similarly exposed as the seals collected in the Bay

2. Stranded, dead animals
- Number unpredictable --17 in 2007/2008 but many likely unusable due to unknown time since death
- Few adults
- How much decomposition is ok?
- Most useful for blubber
- No blood samples

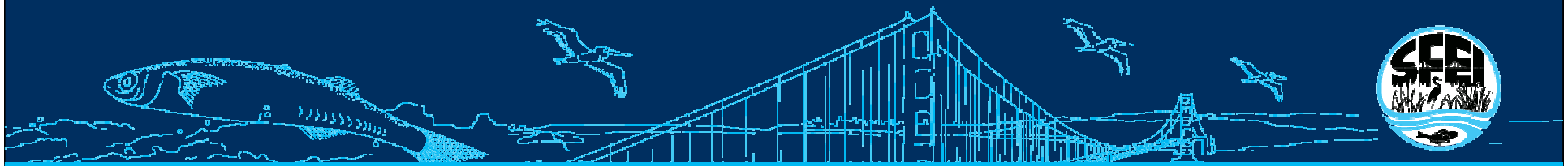
How many samples can we collect?

3. Live captures?

- Increased likelihood of high quality blood samples
- Contract with Jim Harvey at Moss Landing Marine Labs for capture expertise (boats, equipment, personnel, permits to work with live harbor seals)?
- ~$10,000 additional cost
- Samples: blood and max 5 g blubber
- Estimate of 5-10 at Castro Rocks, 5-10 at Mowry Slough

TMMC:

- Could get close to 20 samples of liver and blubber without live captures
- Stranded seals represent unhealthy animals
- Preferable not to use live captures for method development purposes



Sampling Considerations

- Live captures?
- Collect from areas just outside of Golden Gate to increase sample size?
- Collect samples in 2009 and 2010, potentially 2011; oversample and select 'best' for analysis
  - 'Best': - in-Bay seals
    - blood/blubber/liver from same animal
    - best quality (i.e. freshly dead)