# Factors Controlling Methylmercury Production in the South River, VA: Substrate Bioavailabilty and Potentials for Methylation and Demethylation

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# Program

- Pilot program – planned for 1 year, may be expanded
- Goal is to develop tools to address phase II questions in the NRDC – settlement eco-study for South River
  - Specifically:
    - What is the relative bioavailability of different mercury sources to mercury methylation?
    - What is the relative importance of different sites or ecosystems to overall methylmercury production?
    - Is there a predictable microbial community associated with areas of elevated mercury methylation?

# Objective 1: Bioavailability of Mercury for Methylation

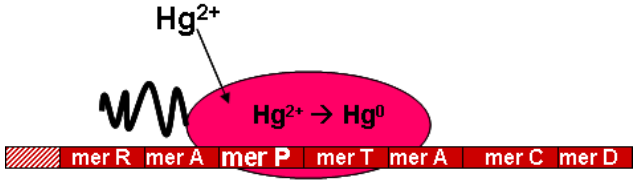
- *Bioavailability experiments*
  - Objectives:
    - Examine the availability of Hg in aqueous samples and water extracts of soils and sediments to microorganisms using a bioreporter approach
    - Compare the bioreporter response with other methods that are commonly used as surrogate assays for Hg bioavailability

- Methods:
  - Compare three experimental procedures for determining bioavailable mercury in a range of South River environmental samples
    - Bio Reporter bacteria
    - "Reactive Hg" assay*
    - Ultrafiltration

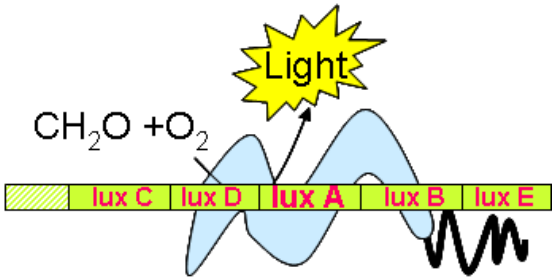
- Substrates to be tested:
  - Water extracts of floodplain soil
  - Porewater from near bank sediments
  - Water extracts of near bank sediment
  - Water extract of the fine silt-clay fraction of main river bed deposits
  - Hyporheic water from gravel bars

* Standard method quantifying Hg pool that is reduced to $Hg^0$ with the addition of stannous citrate

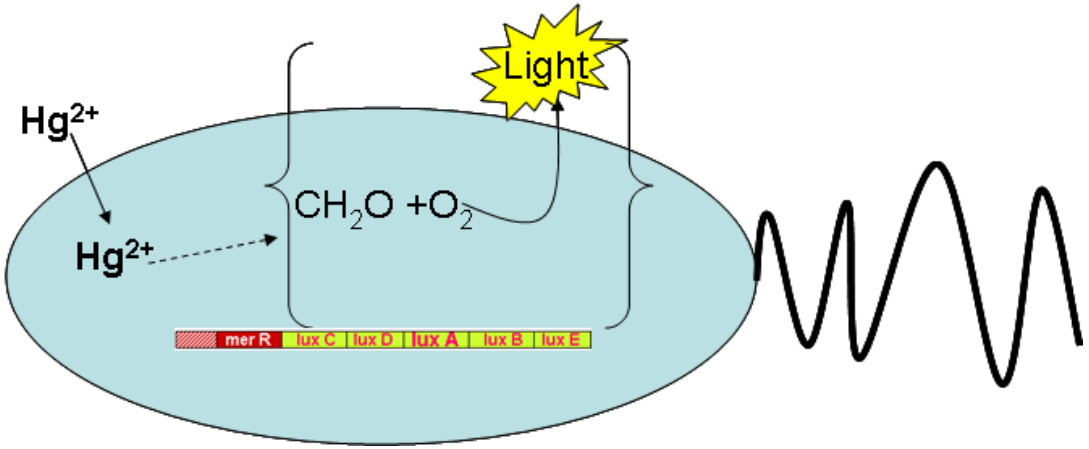
# The BioReporter Concept




**Mer genes found on transposable elements in nature reduce intracellular Hg to $Hg^0$. Expression is induced by the presence of $Hg^{2+}$**




***Vibrio fisheri* – a luminescent bacterium produces light from organic electron donor and Oxygen (lux genes)**




**BioReporter – Mer gene promoter and first structural gene attached to lux structural genes and introduced into host bacterium. This results in a bacterial strain that emits light proportional to intracellular mercury concentrations**

# Objective 2: Potential Rates of Mercury Methylation and MethyMercury Demethylation

- *Determinations of potential rates of methylation and demethylation*
  - Objectives:
    - Determination of South River ecosystem compartments likely to produce MeHg
    - Correlation of methylation and demethylation potentials to microbial communities (see objective 3)

- Methods:
  - Quantify rates of
    - Methylation of $^{203}HgCl$ (track production of organic $^{203}Hg$)
    - Demethylation of $^{14}C$-MeHg (track production of $^{14}CO_2$ and $^{14}CH_4$)

- Substrates to be tested:
  - flood plain soils
  - near bank sediments
  - fine silt-clay riverbed deposits

# Objective 3: Microbial Community Analysis

- *Microbial community analysis*
  - Objective:
    - Examination of the structure of the microbial communities in South River samples and correlate to mercury methylation potential.

- Methods:
  - Extract DNA from environmental samples showing high potential for Hg methylation
  - Construct and sequence16S rRNA gene clone libraries
  - Identify dominant organisms
  - Compare results to methylation/demethylation potentials:

- Substrates to be tested:
  - flood plain soils
  - near bank sediments
  - fine silt-clay riverbed deposits