

H4IIE Bioassay and EROD:

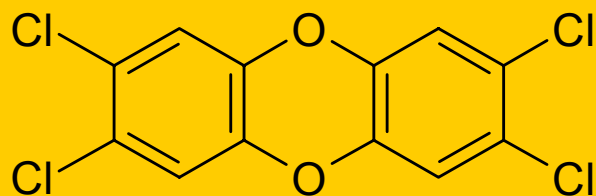
Methods, Performance, and Relevance

Columbia Environmental Research Center

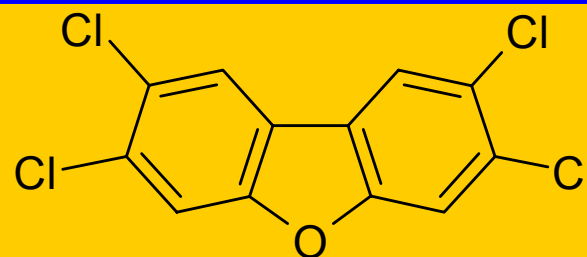
U.S. Geological Survey

4200 New Haven Road

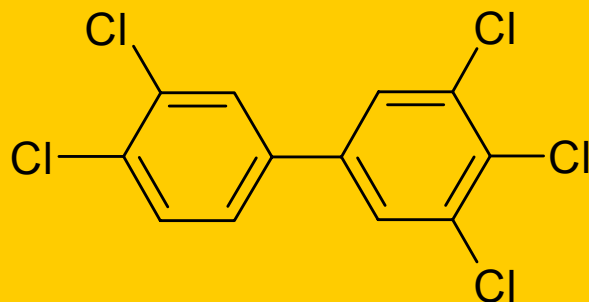
Columbia, MO 65201



(a) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin



(b) 2,3,7,8-Tetrachlorodibenzofuran



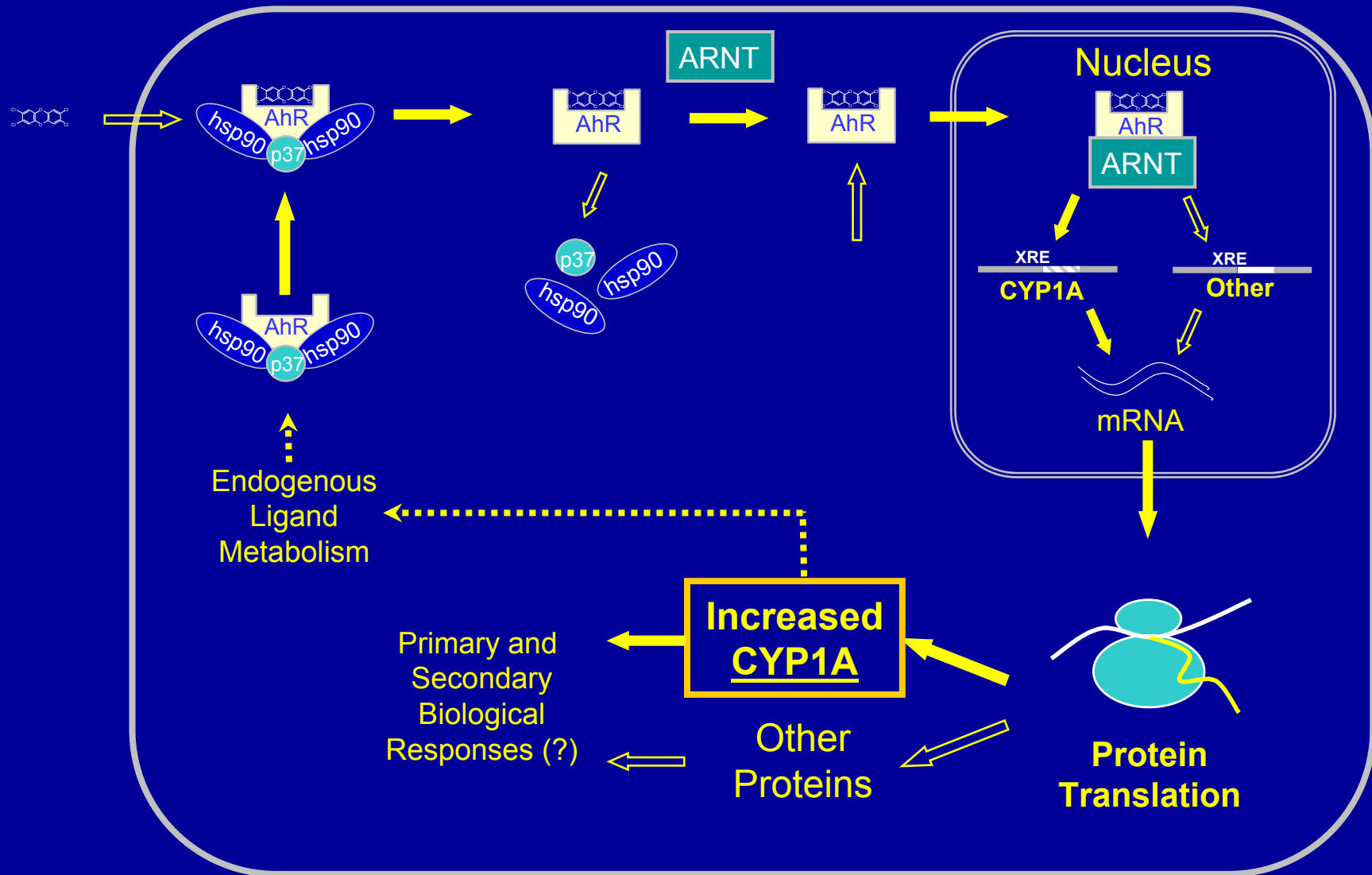
(c) 3,3',4,4',5-Pentachlorobiphenyl (PCB 126)

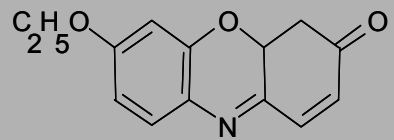


(d) Benzo[a]pyrene

Representative AhR ligands. The molecules demonstrate the general structure of compounds in the following classes: (a) polychlorinated dibenzo-*p*-dioxins (PCDDs), (b) polychlorinated dibenzofurans (PCDFs), (c) polychlorinated biphenyls (PCBs) and (d) polycyclic aromatic hydrocarbons (PAHs).

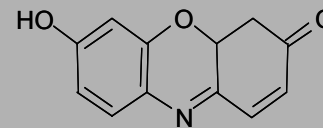
Mechanism of CYP1A Induction





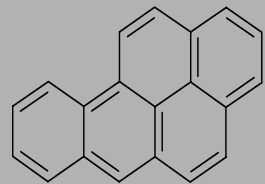
7-Ethoxyresorufin

*7-Ethoxyresorufin
O-deethylase (EROD)*



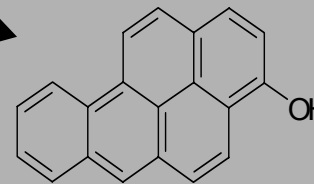
Resorufin
550 nm/585 nm

Cytochrome
P4501A1



Benzo[a]pyrene (BaP)

*Aryl hydrocarbon
hydroxylase (AHH)*



3-Hydroxybenzo[a]pyrene
396 nm/522 nm

+
other
metabolites

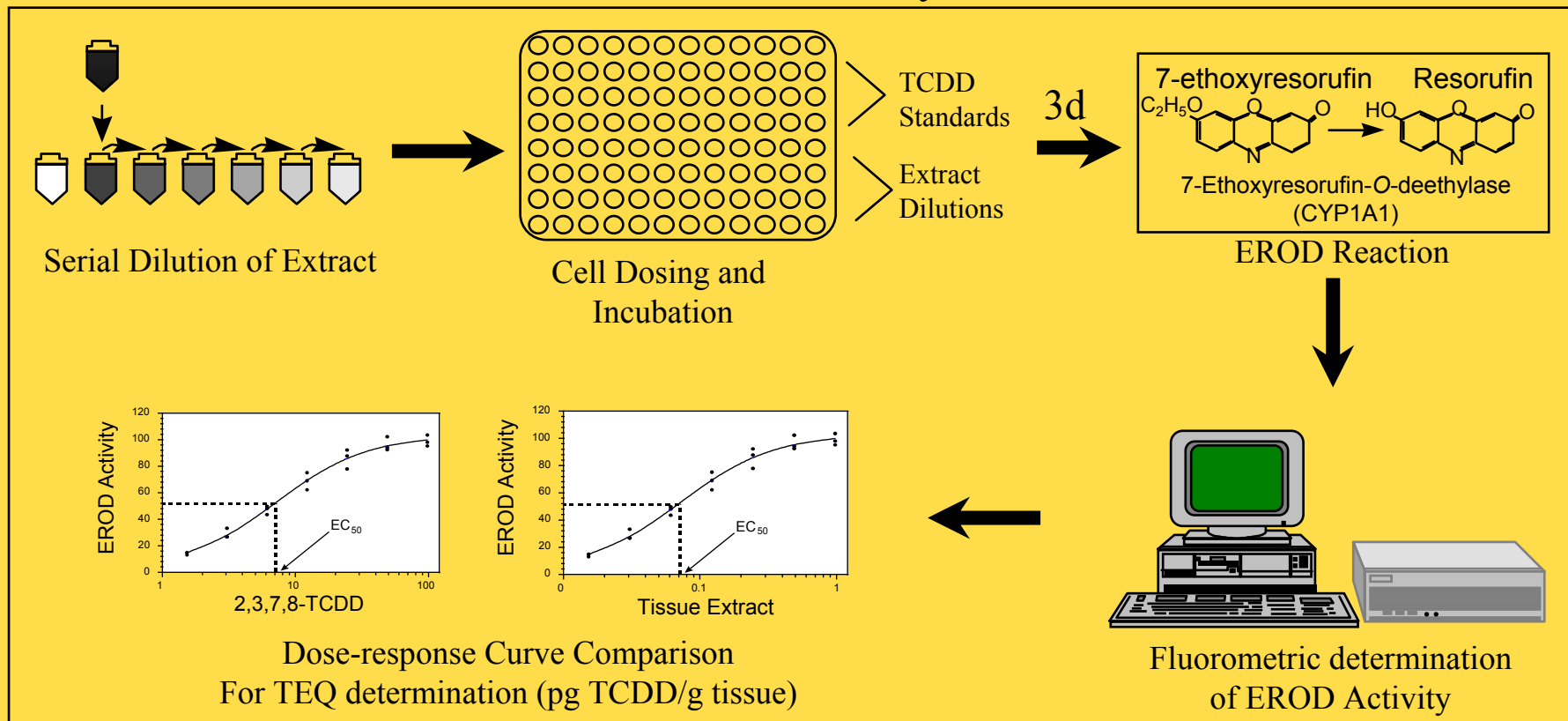
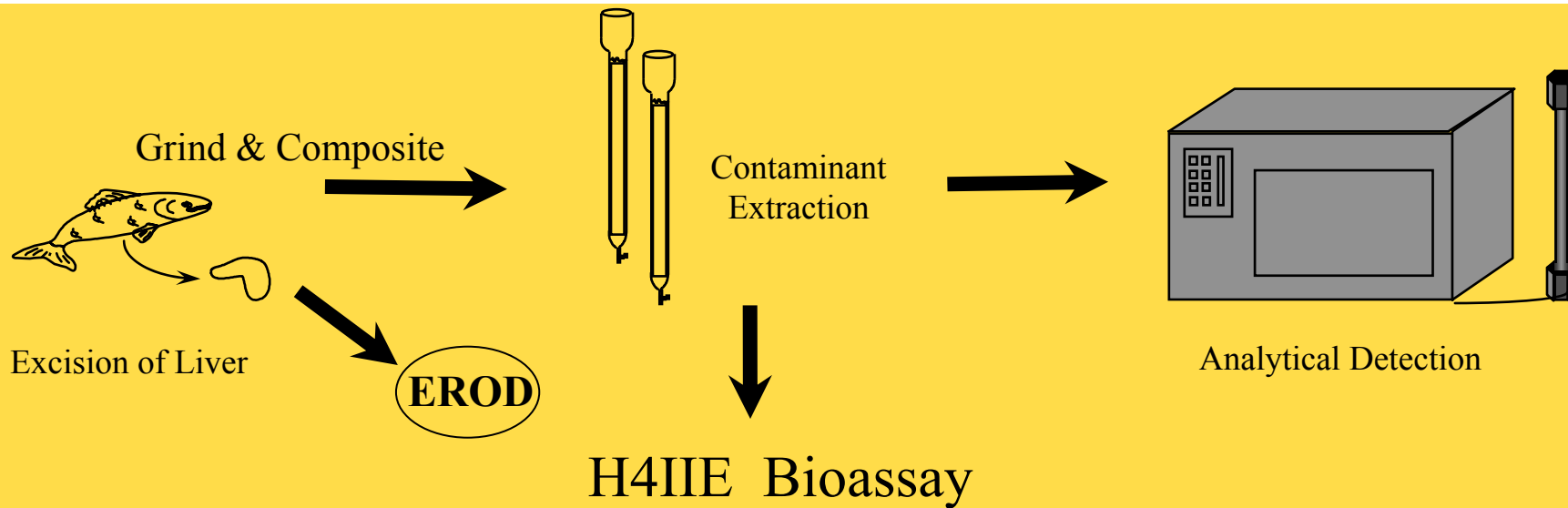
EROD and H4IIE Comparison

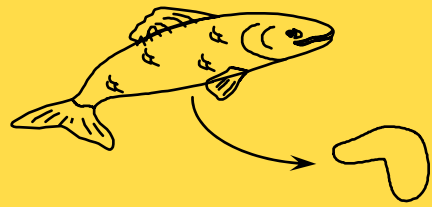
- EROD

- Measurement of CYP1A in the fish
- Species specific
- Exposure in the field
 - PCBs, Dioxins, Furans, and PAHs
- Susceptible to degradation***

- H4IIE bioassay

- Chemicals taken from fish
- Extraction removes PAHs
- Controlled exposure of H4IIE cells in lab
- CYP1A measurement endpoint





Excision of Liver

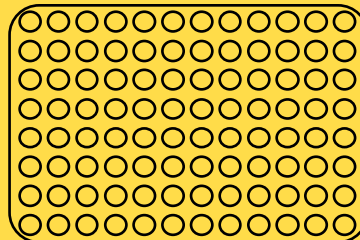
-80 C

Centrifuge and prepare
microsomes



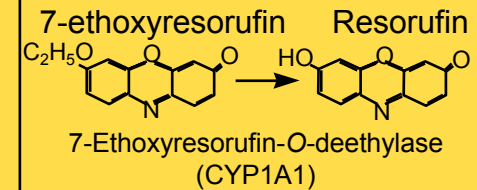
EROD assay

Microsomal preparation
- CYP1A protein



Add microsomes and
other reagents

10-20 min.



EROD Reaction



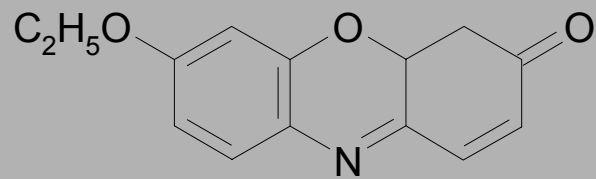
Fluorometric determination
of EROD Activity

EROD rate is determined (pmol resorufin formed/min/g tissue)

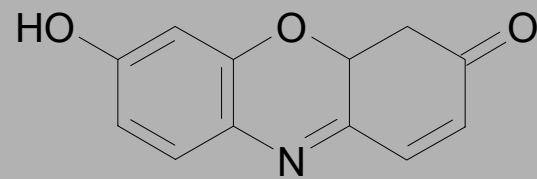
- catalytic activity
- reflective of amount of protein (CYP1A)
- indicative of exposure to PHHs/PAHs



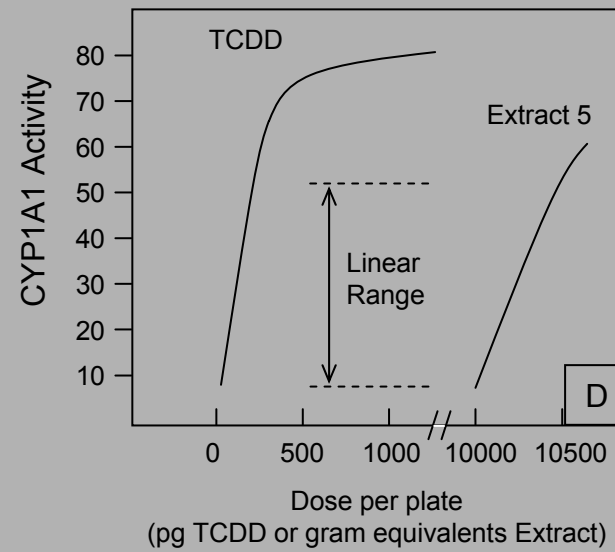
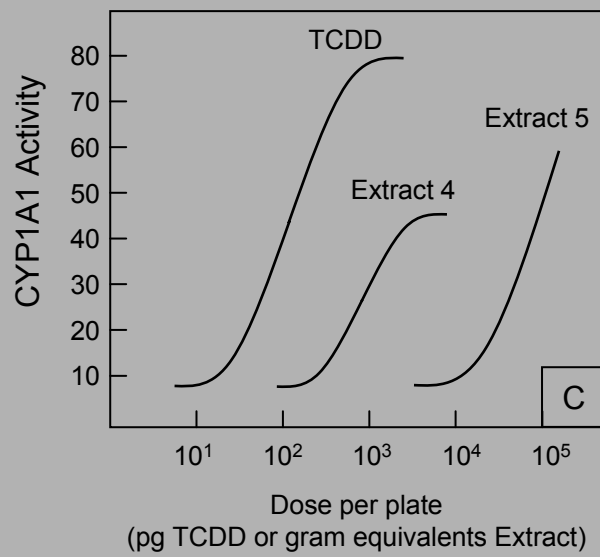
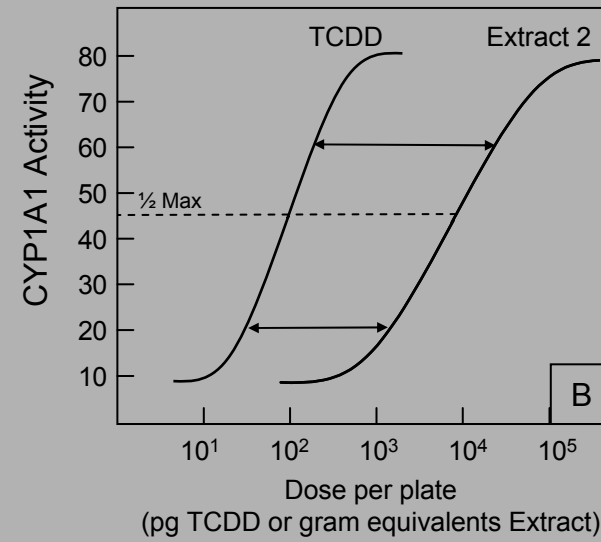
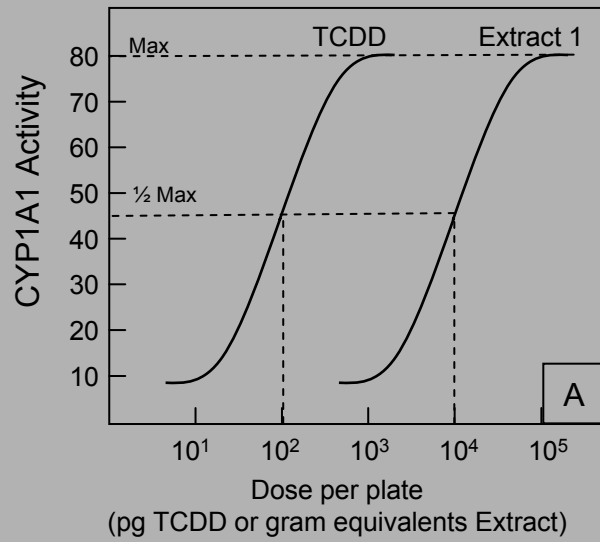
7-Ethoxyresorufin



Resorufin



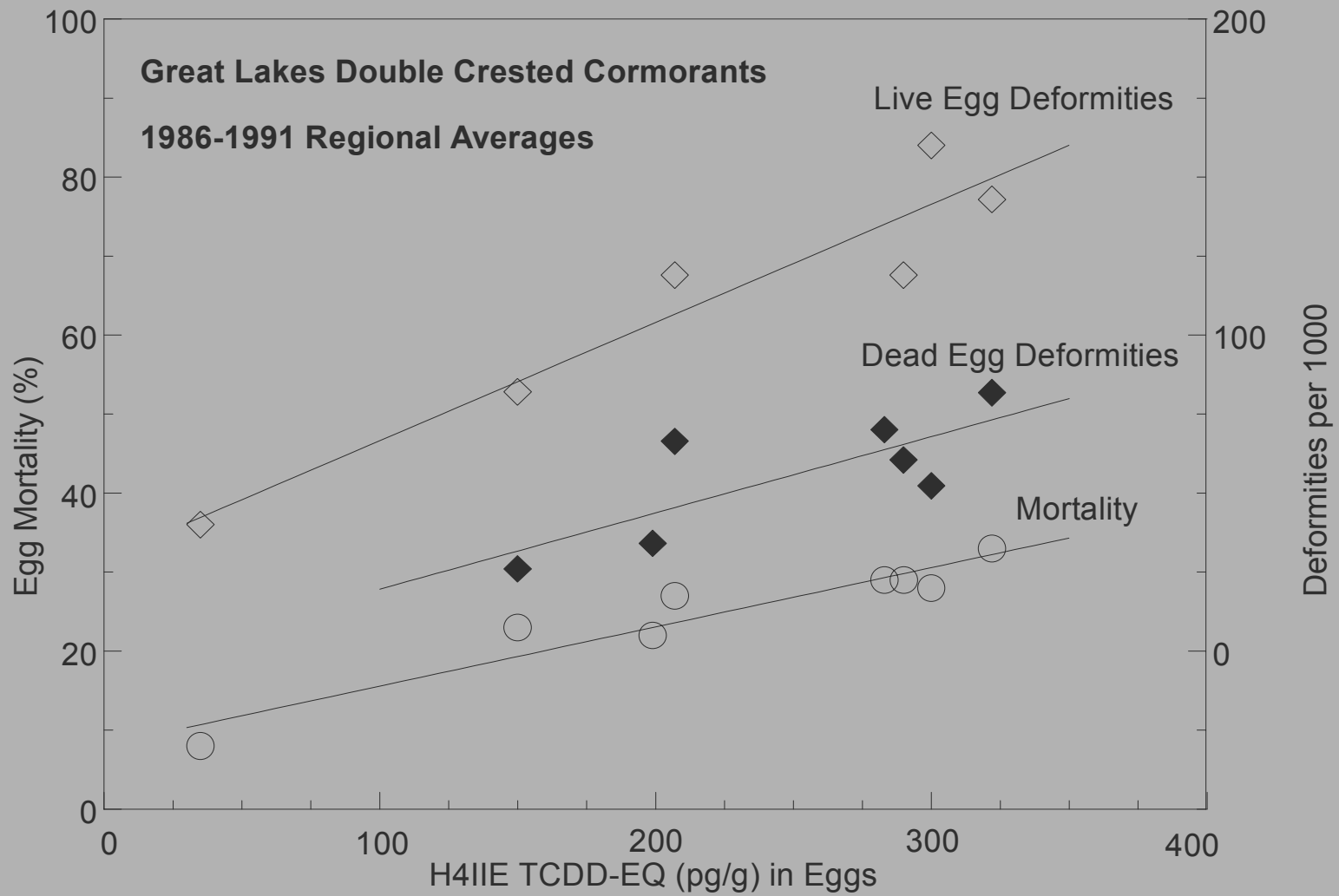
7-Ethoxyresorufin-O-deethylase
(CYP1A)



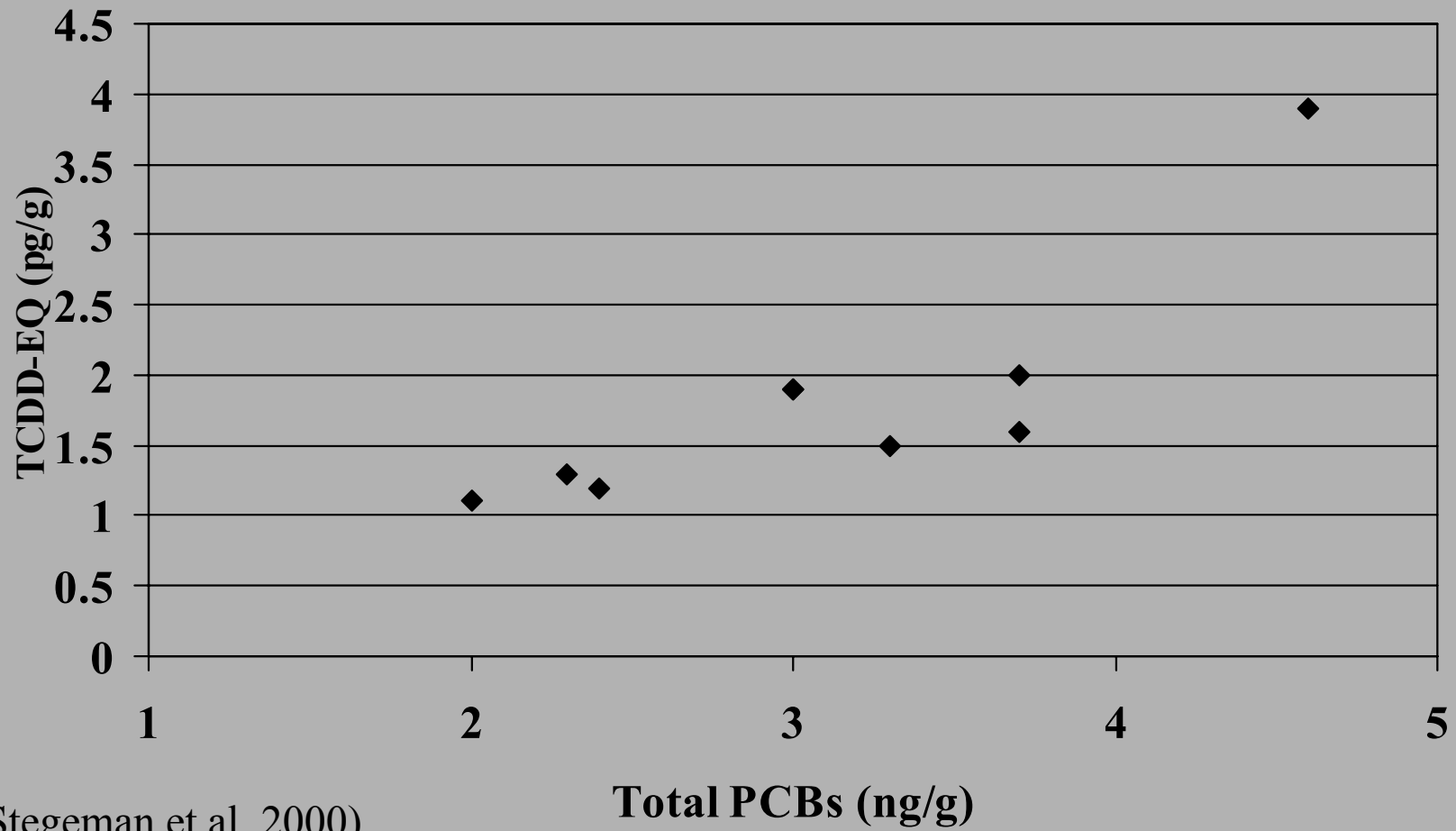
Characteristics of PLHC-1 and H4IIE Hepatoma Cell Lines

- Basal EROD 0.5 - 1.0 pmol/min/mg
- Limit of Detection 100 fg TCDD (0.3 fmol, 1.3 pM)
- Median Response (ED50) 3 pg TCDD (10 fmol, 38 pM)
- Maximal Induction 100 - 250 pmol/min/mg
(100-400 fold)

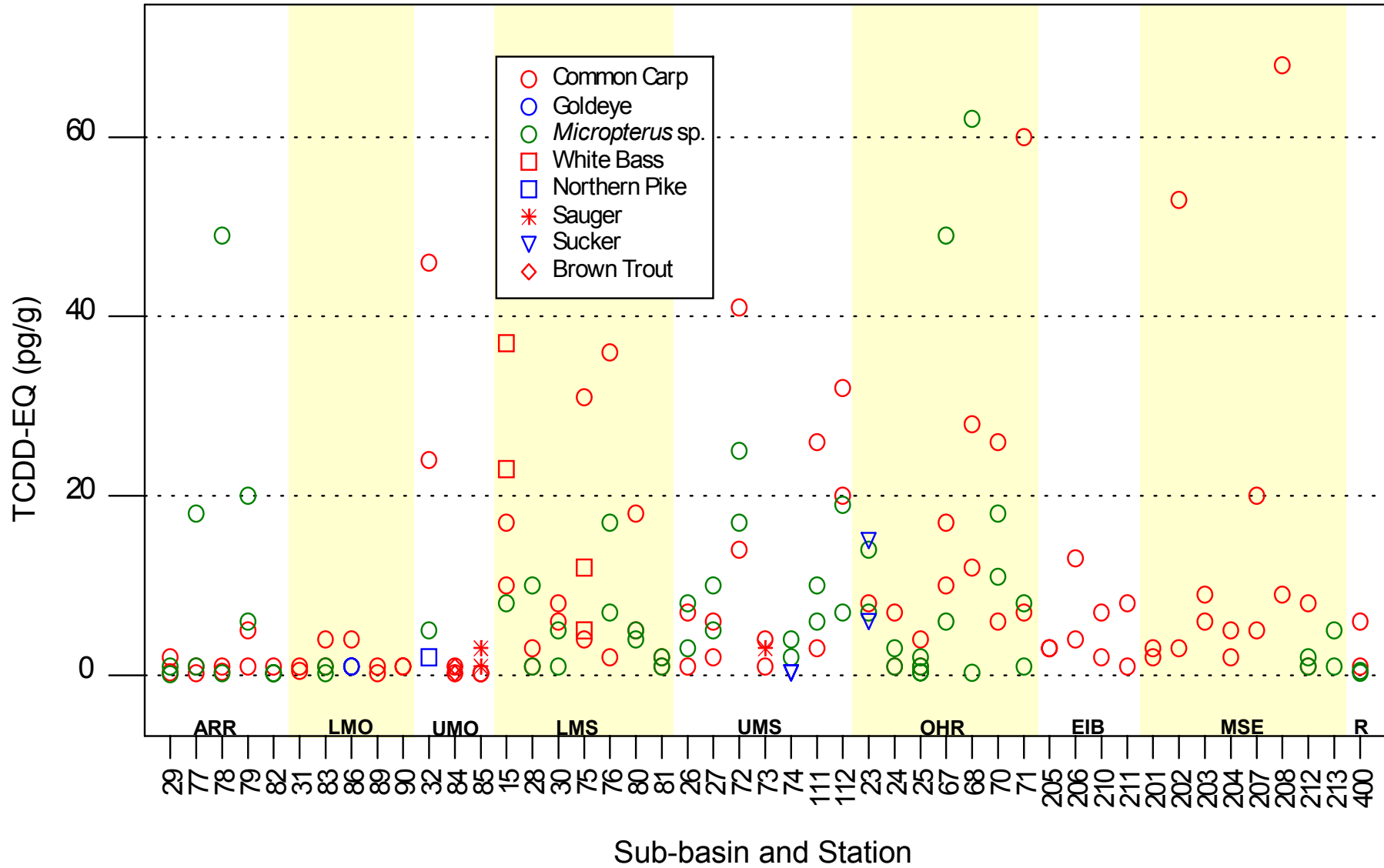
(Tillitt, et al. 1992; Ludwig et al. 1996)

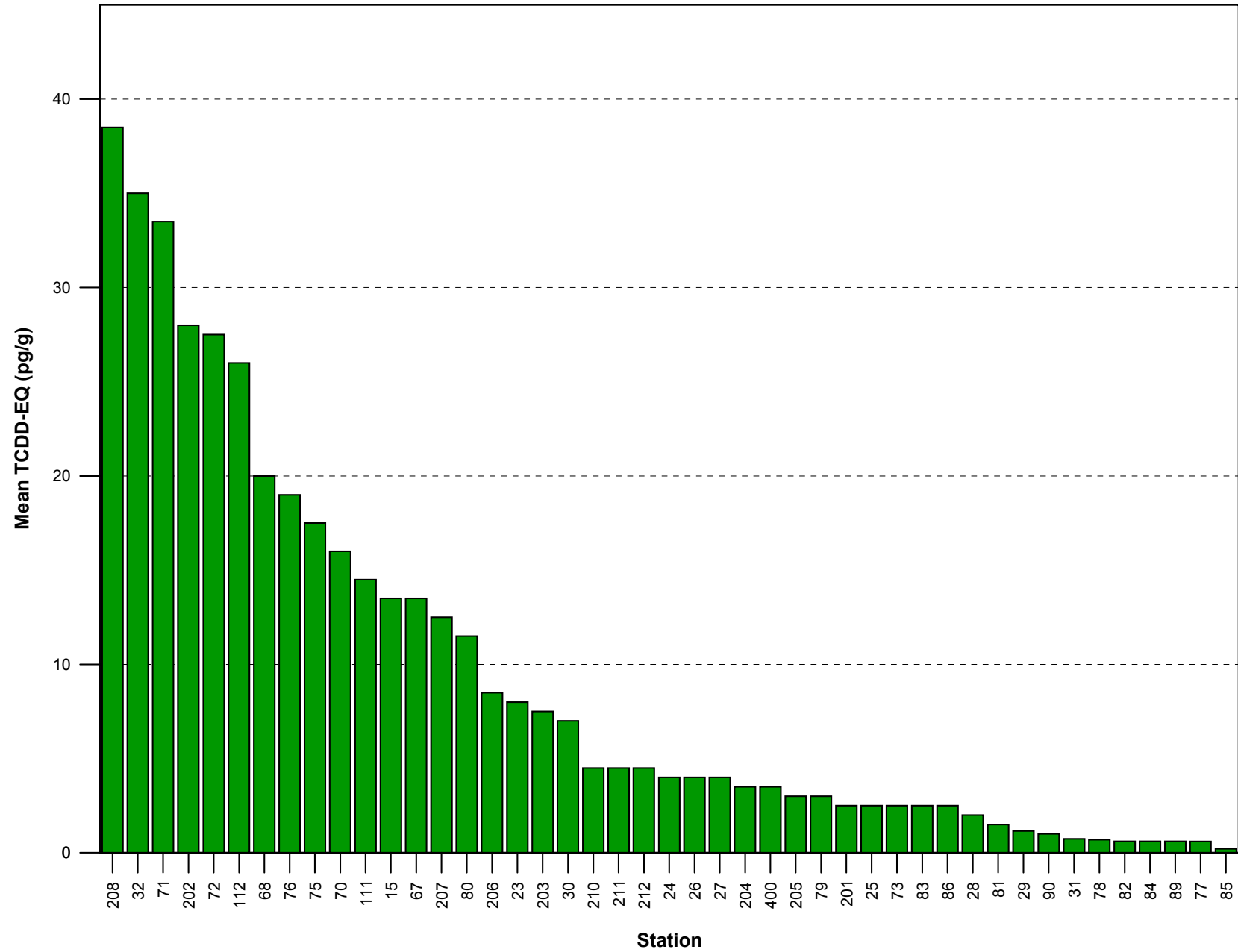


PLHC-1 Bioassay TCDD-EQ (pg/g) versus Total PCBs (ng/g)
in Mid-Water Fishes from the Atlantic Ocean.

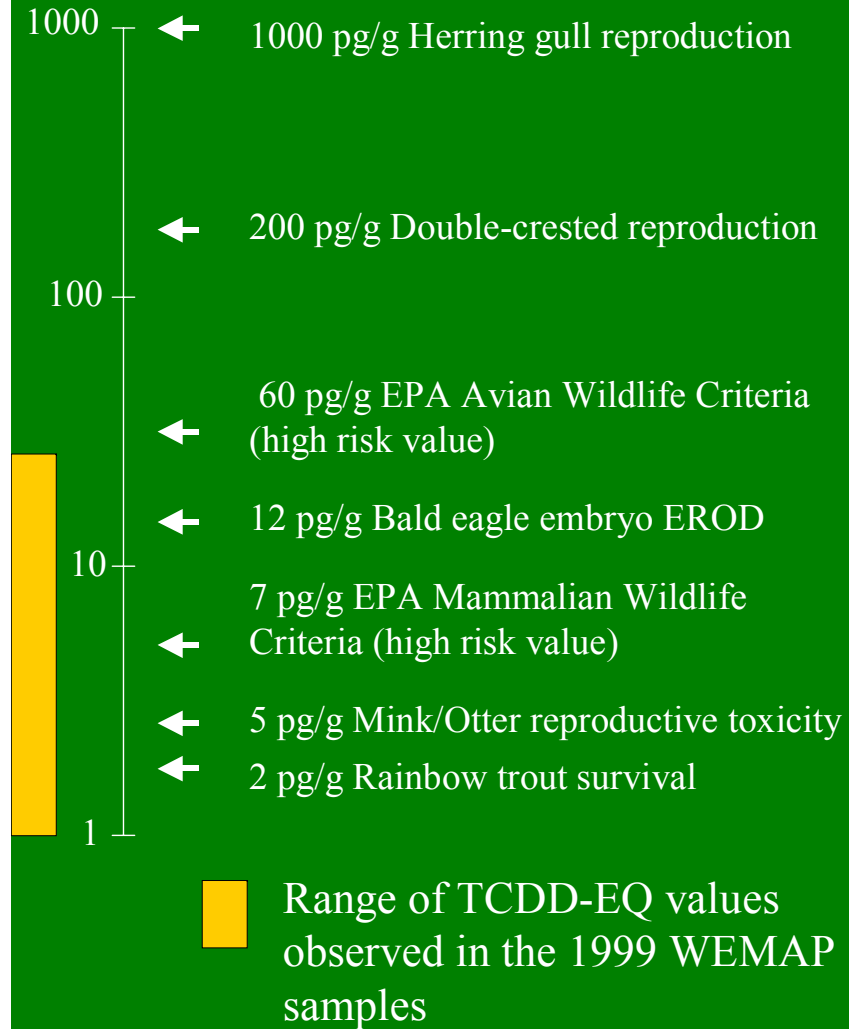


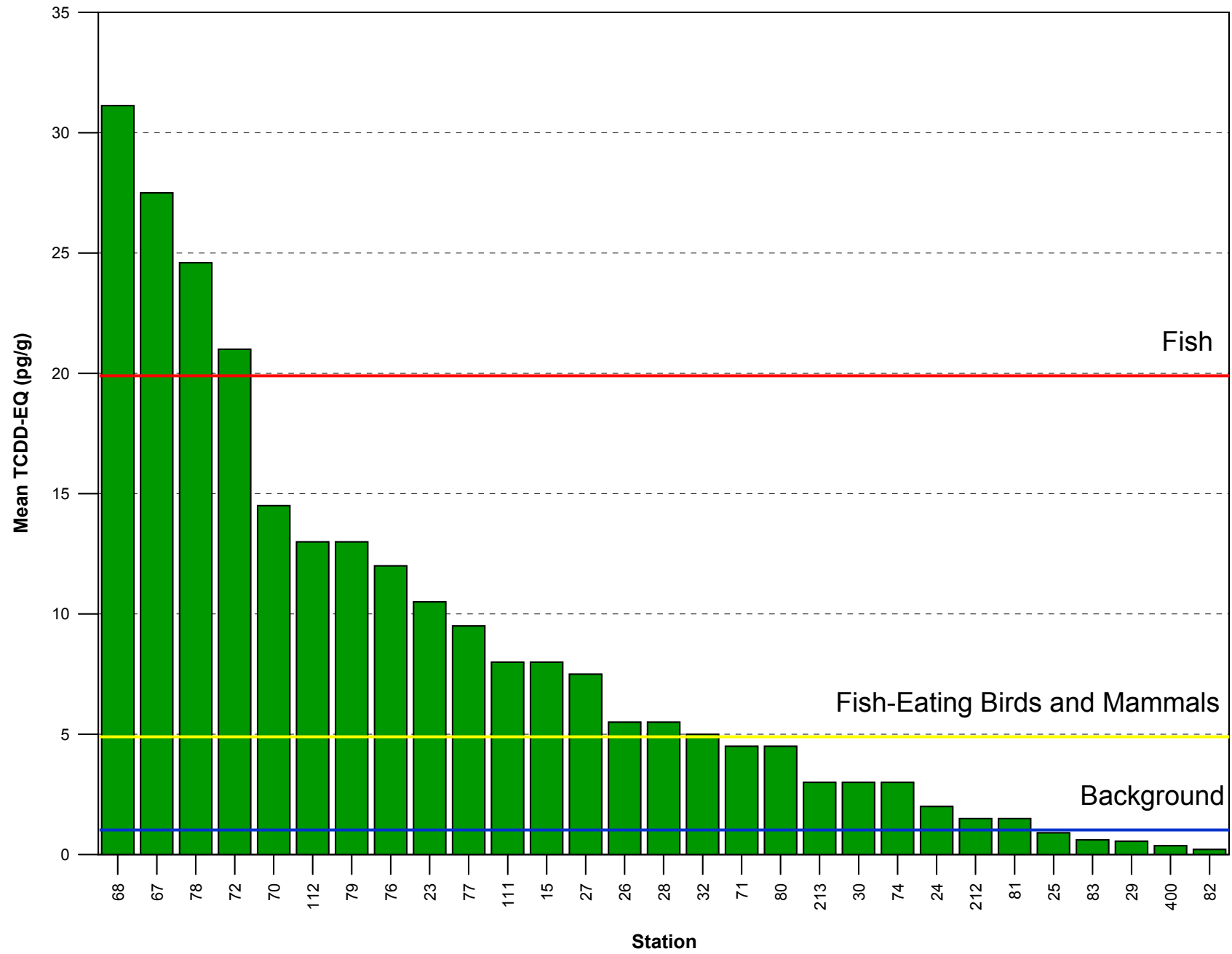
(Stegeman et al. 2000)

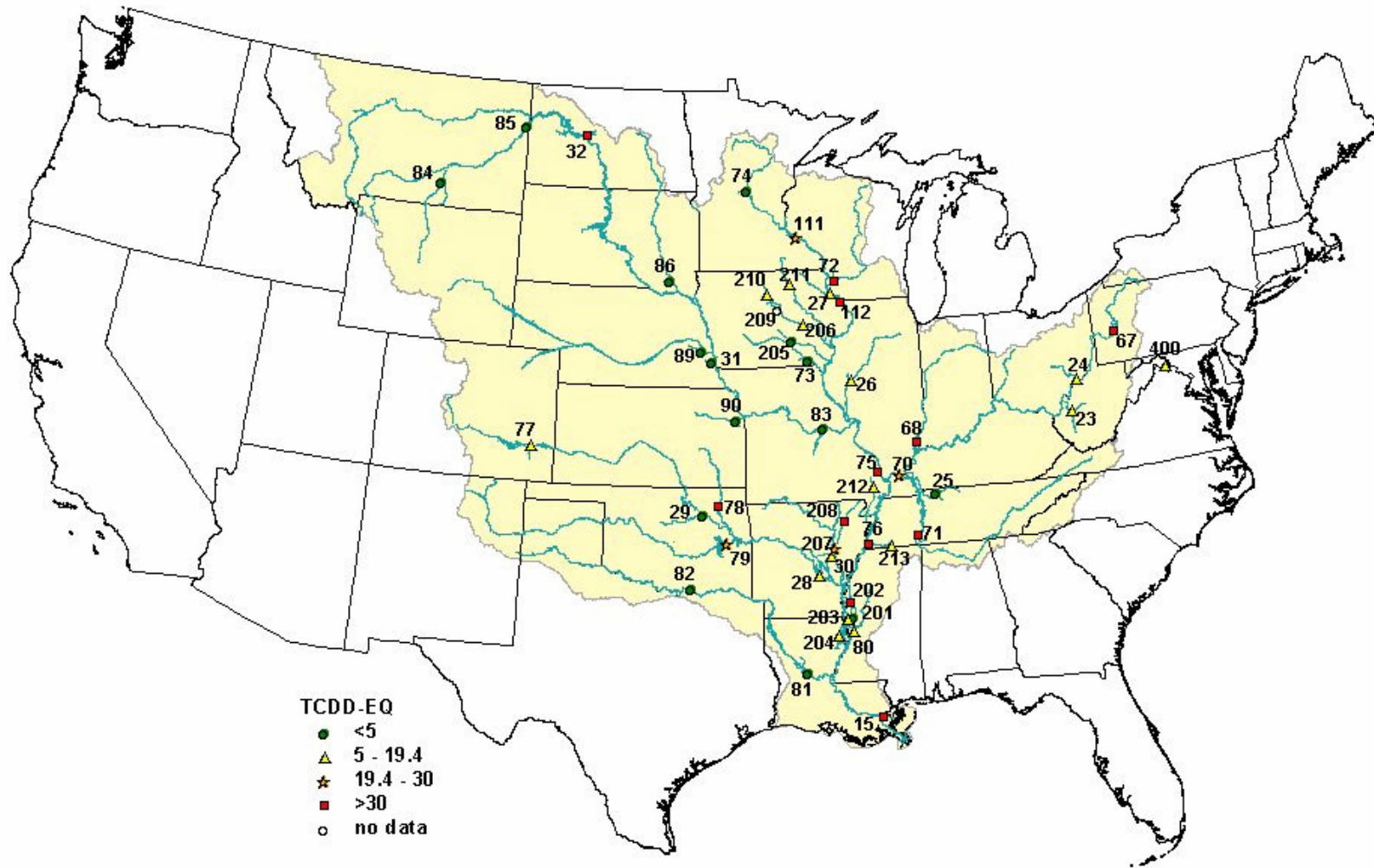




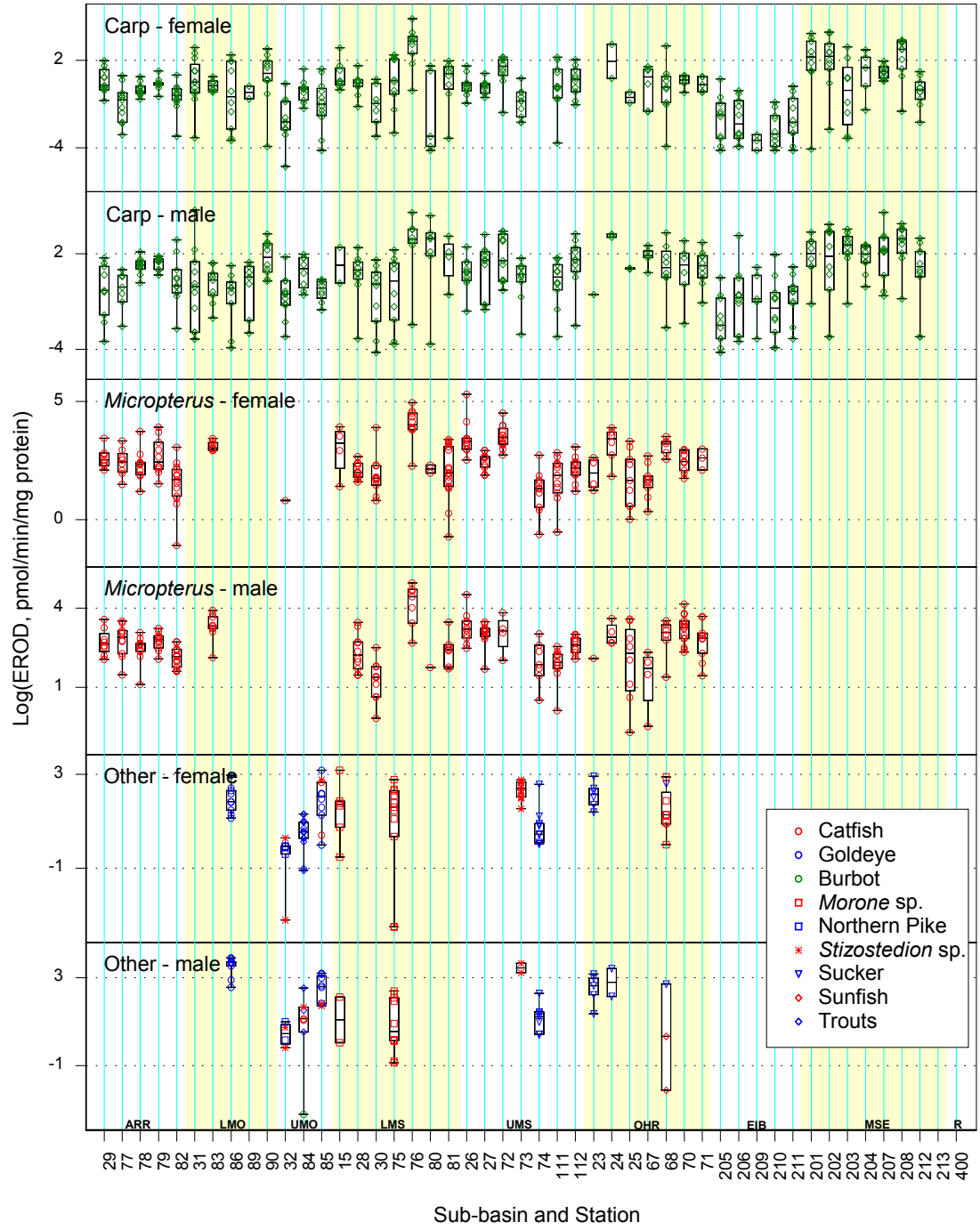
Dietary Thresholds for TCDD or TEQ Toxicity in Fish and Wildlife.



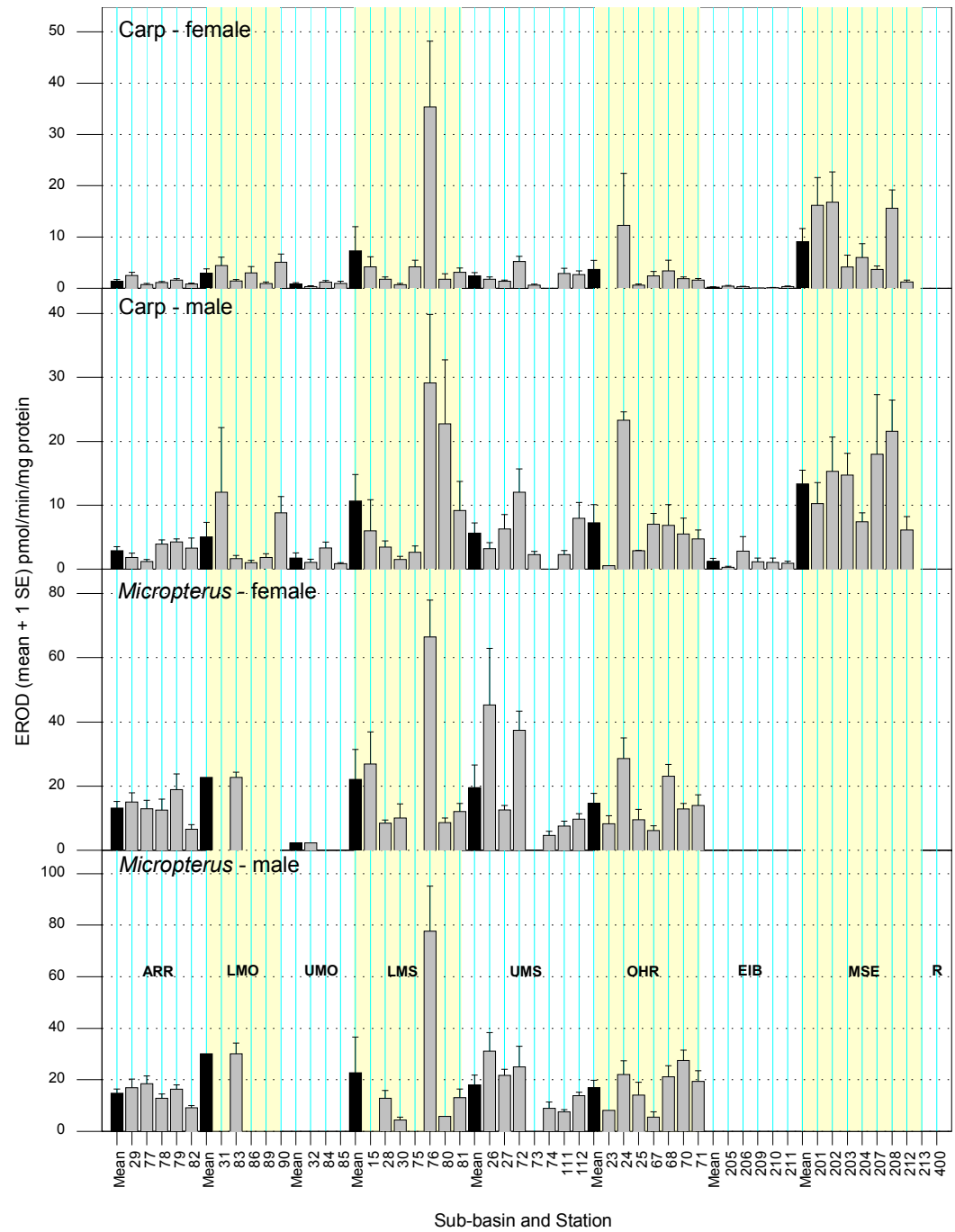


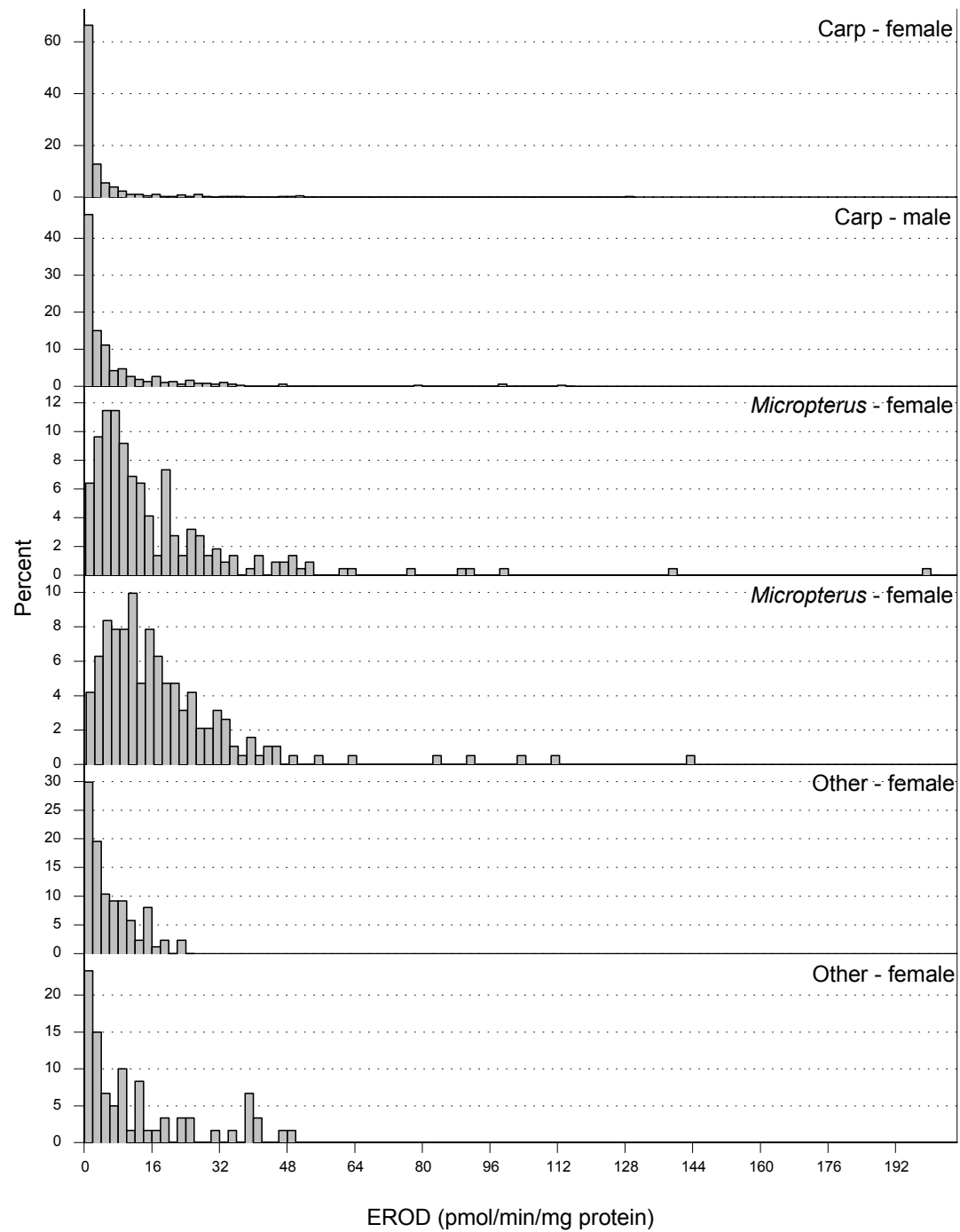


● <5
 ▲ 5 - 19.4
 ★ 19.4 - 30
 ■ >30
 ○ no data

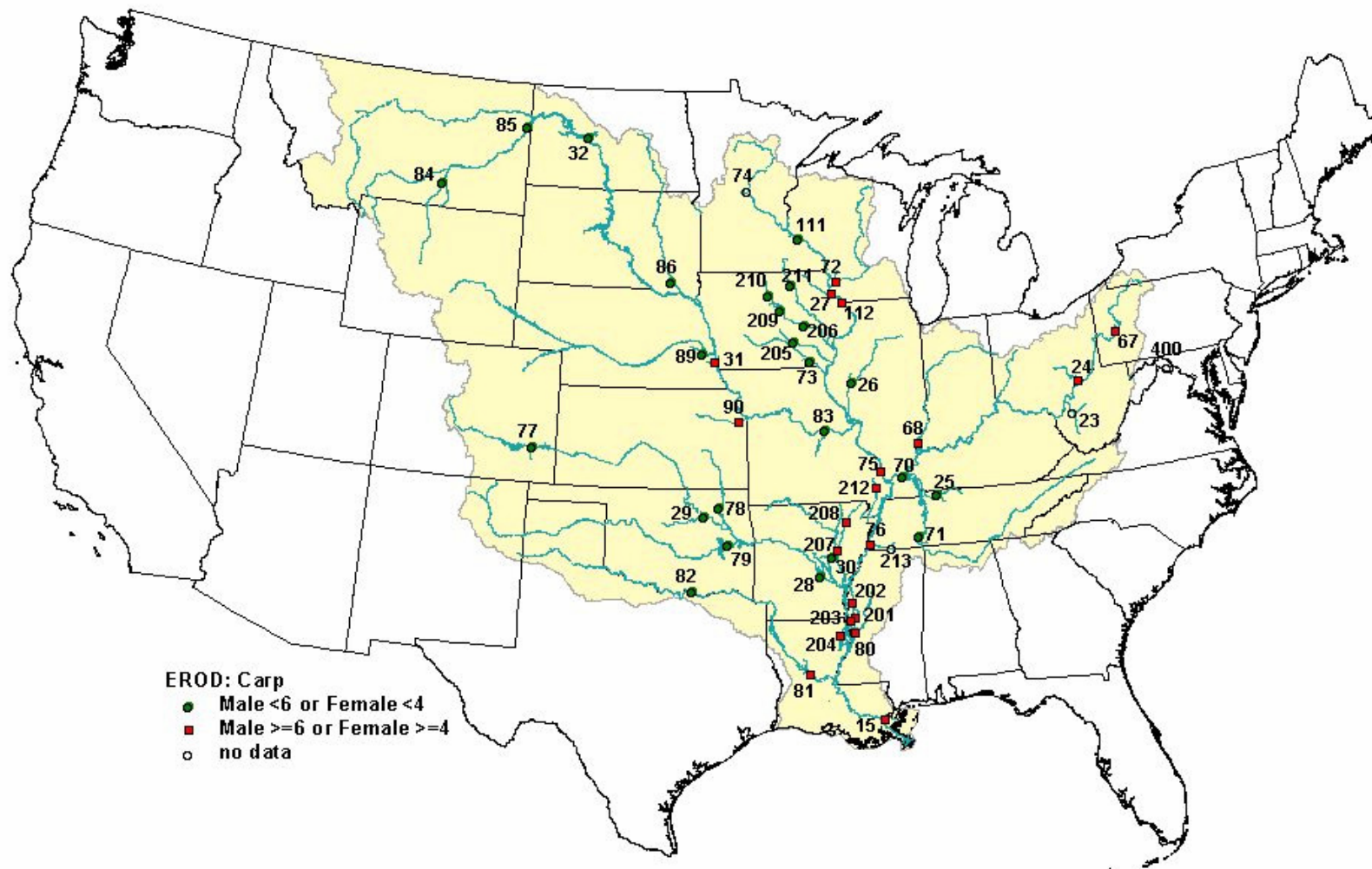


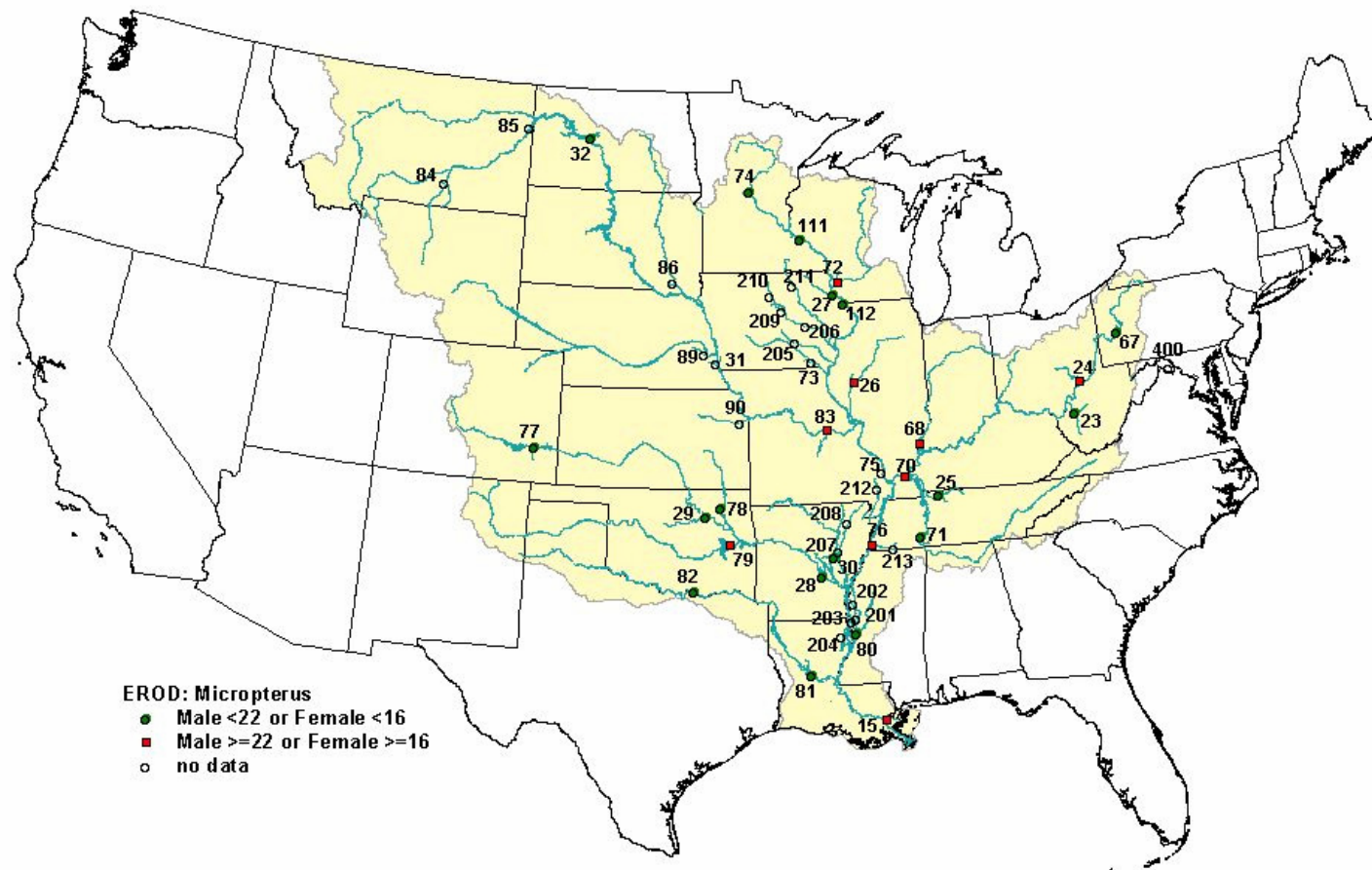
Sub-basin and Station

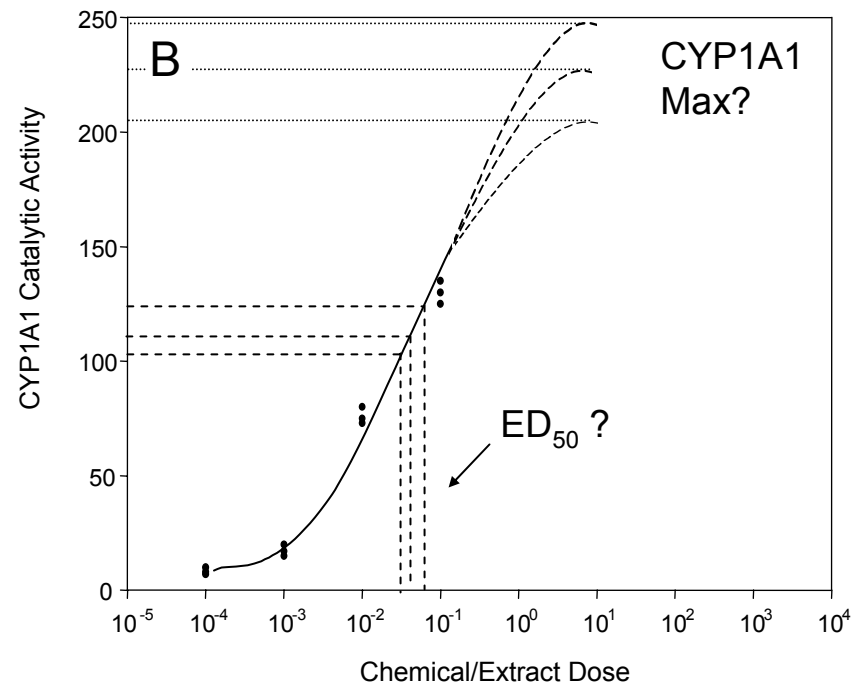
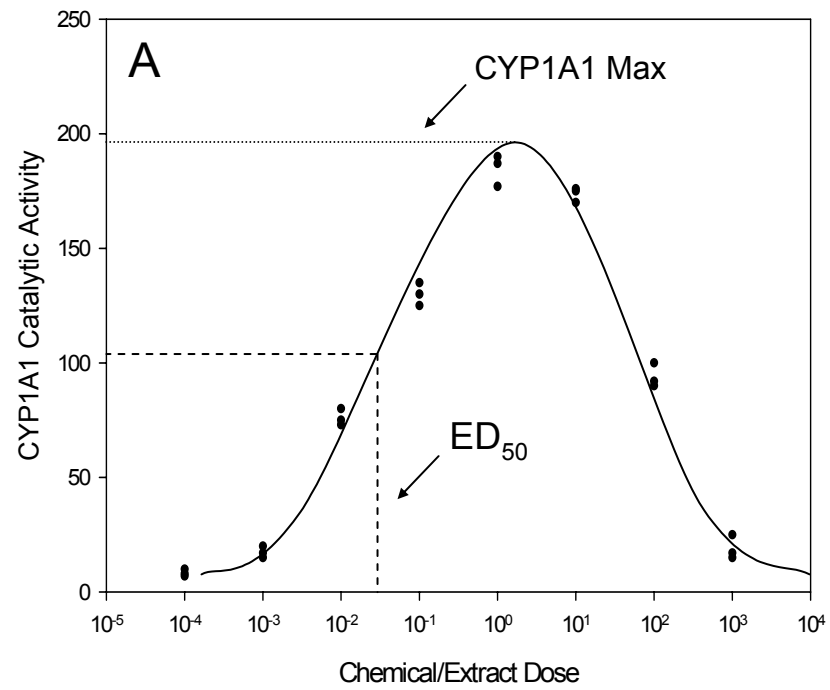




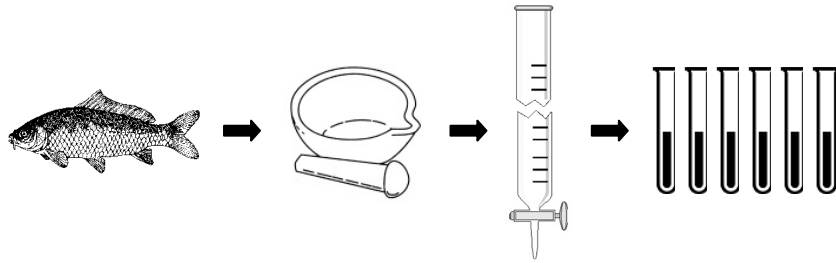
EROD (pmol/min/mg protein)



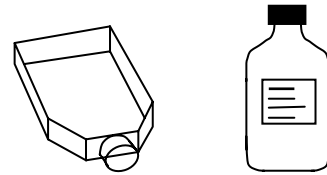




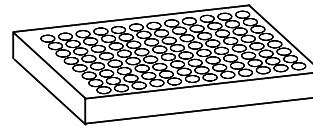
1. Chemical Extract Preparation



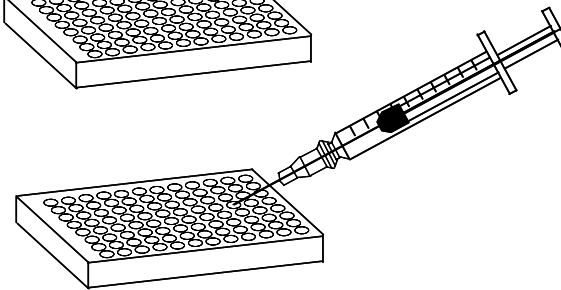
2. H4IIE Cell Culture



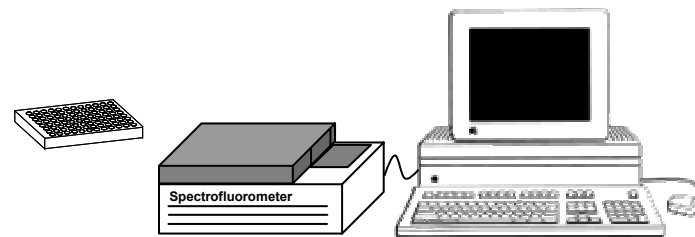
3. Plating

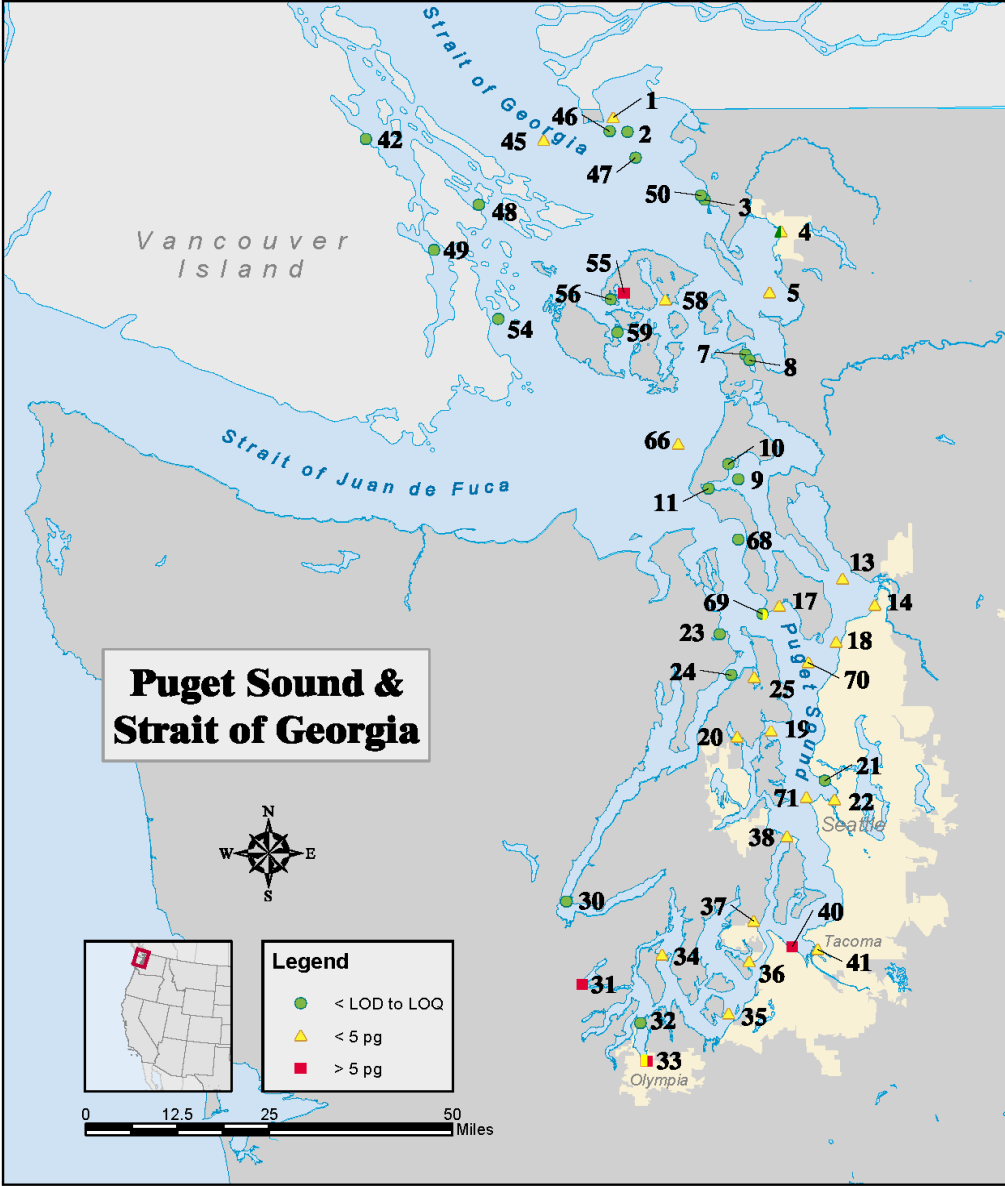


4. Dosing and Incubation

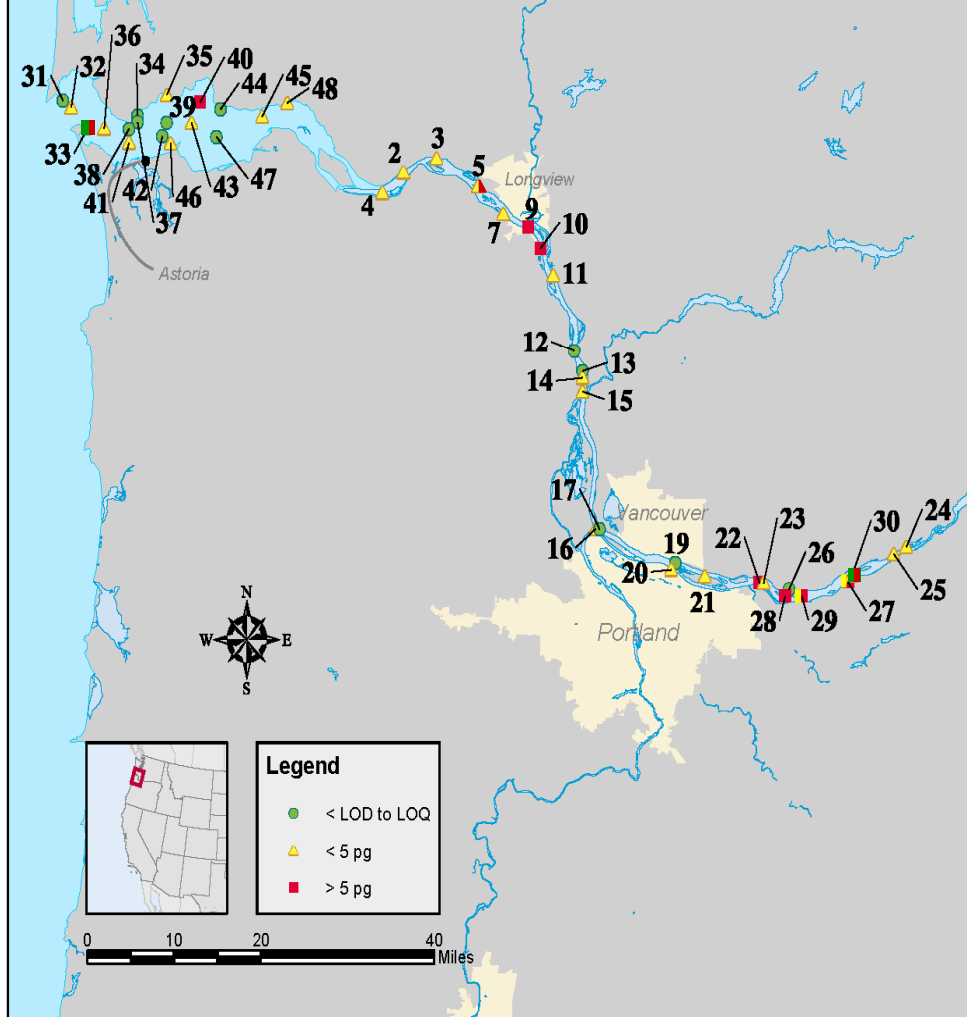


5. CYP1A1 Catalytic Activity (EROD or AHH)



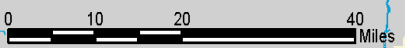


Columbia River (Lower & Mouth)



Legend

- < LOD to LOQ
- ▲ < 5 pg
- > 5 pg



San Francisco Bay

