Use of Bioassays for Identifying Endocrine Disruptors in the Environment

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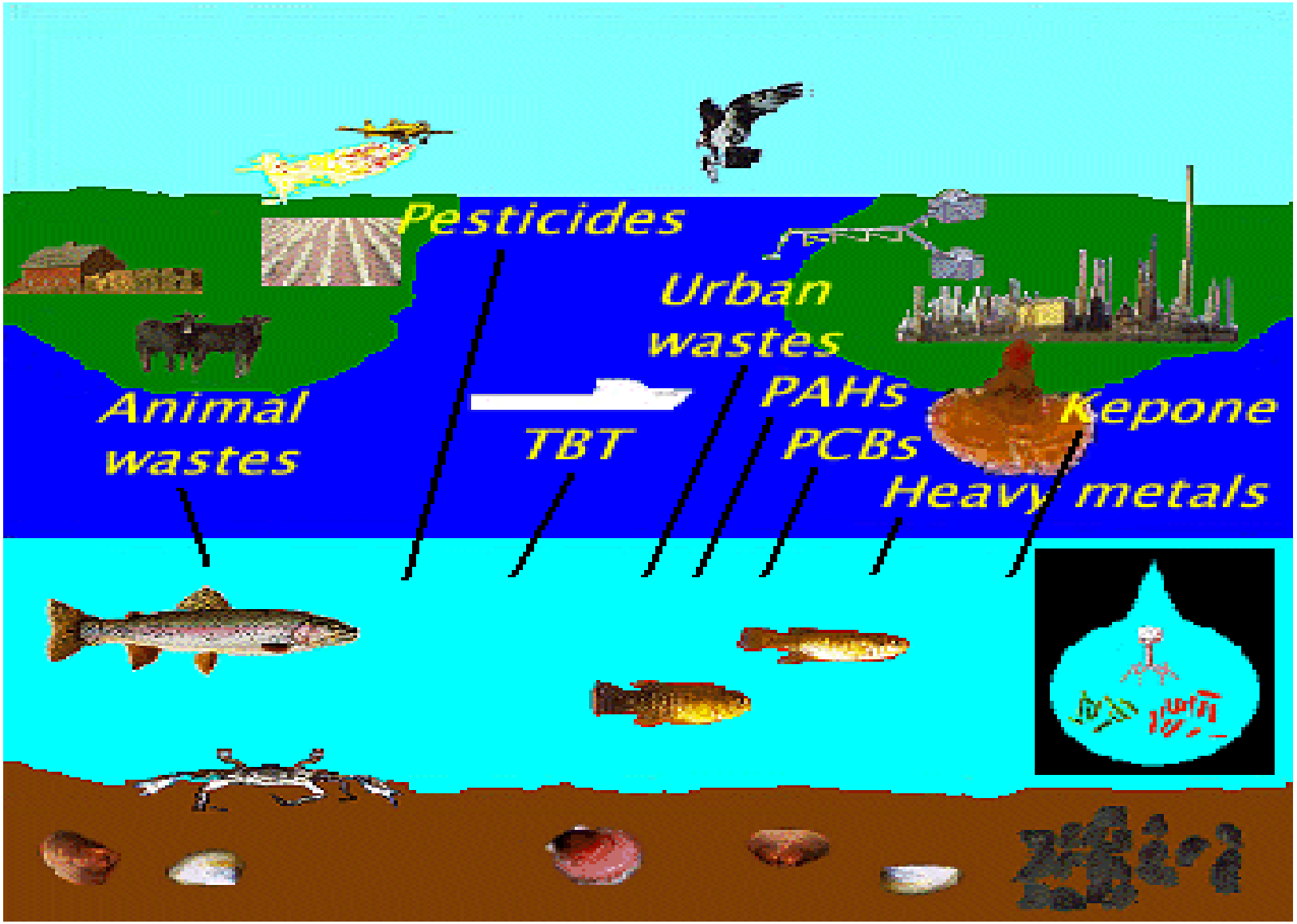

Pesticides
Urban wastes
Animal wastes
TBT
PAHs
PCBs
Kepone
Heavy metals

What is a Contaminant of Emerging Concern?

- Chemicals and other substances that have no regulatory standard
- Chemicals that have been recently "discovered" in natural streams (often because of improved analytical chemistry detection levels), and potentially cause deleterious effects in aquatic life at environmentally relevant concentrations.
- They are pollutants not currently included in routine monitoring programs and may be candidates for future regulation depending on
  - Their (eco)toxicity
  - Potential health effects
  - Public perception
  - Frequency of occurrence in environmental media.
- CECs are not necessarily new chemicals. They include pollutants that have often been present in the environment, but whose presence and significance are only now being evaluated.

USEPA 2008

Examples of CECs

- Personal Care Products (sunscreens;perfume/musks)
- Pharmaceuticals (antibiotics/antimicrobials; B-blockers)
- Industrial (Detergents- APEs)
- Agricultural(Current Use Pesticides-pyrethroids/fipronil)
- Natural Hormones
- Food additives and constituents (phytoestrogens, caffeine, sweeteners)
- Transformation Products
- Inorganic constituents (boron, perchlorate)
- Nanomaterials

Anderson et al. 2010

“……because as we know, there are known knowns;
there are things we know we know.

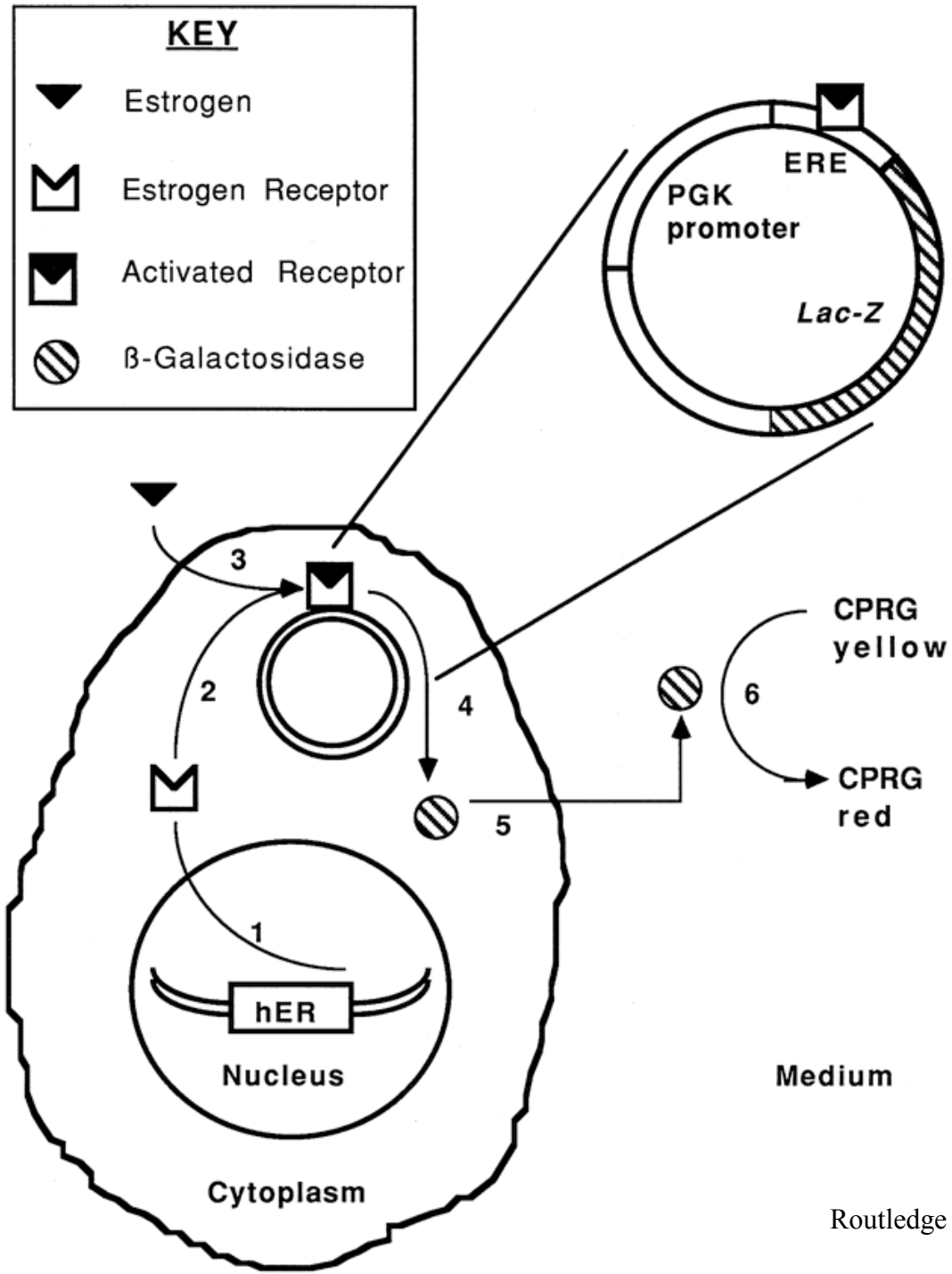
We also know there are known unknowns;
that is to say we know there are some things we do not know.

But there are also unknown unknowns -- the ones we don't know we don't know”

How to Measure CECs?

- Analytical Chemistry
  - Bioavailability?
  - Unknowns?
- Bioassay
  - In vitro (Receptor driven--Cell lines)
  - In vivo (Receptor driven--whole animal)

Yeast Estrogen Screen



Routledge and Sumpter 1996

Example of EEQ Concentration-Response Curve

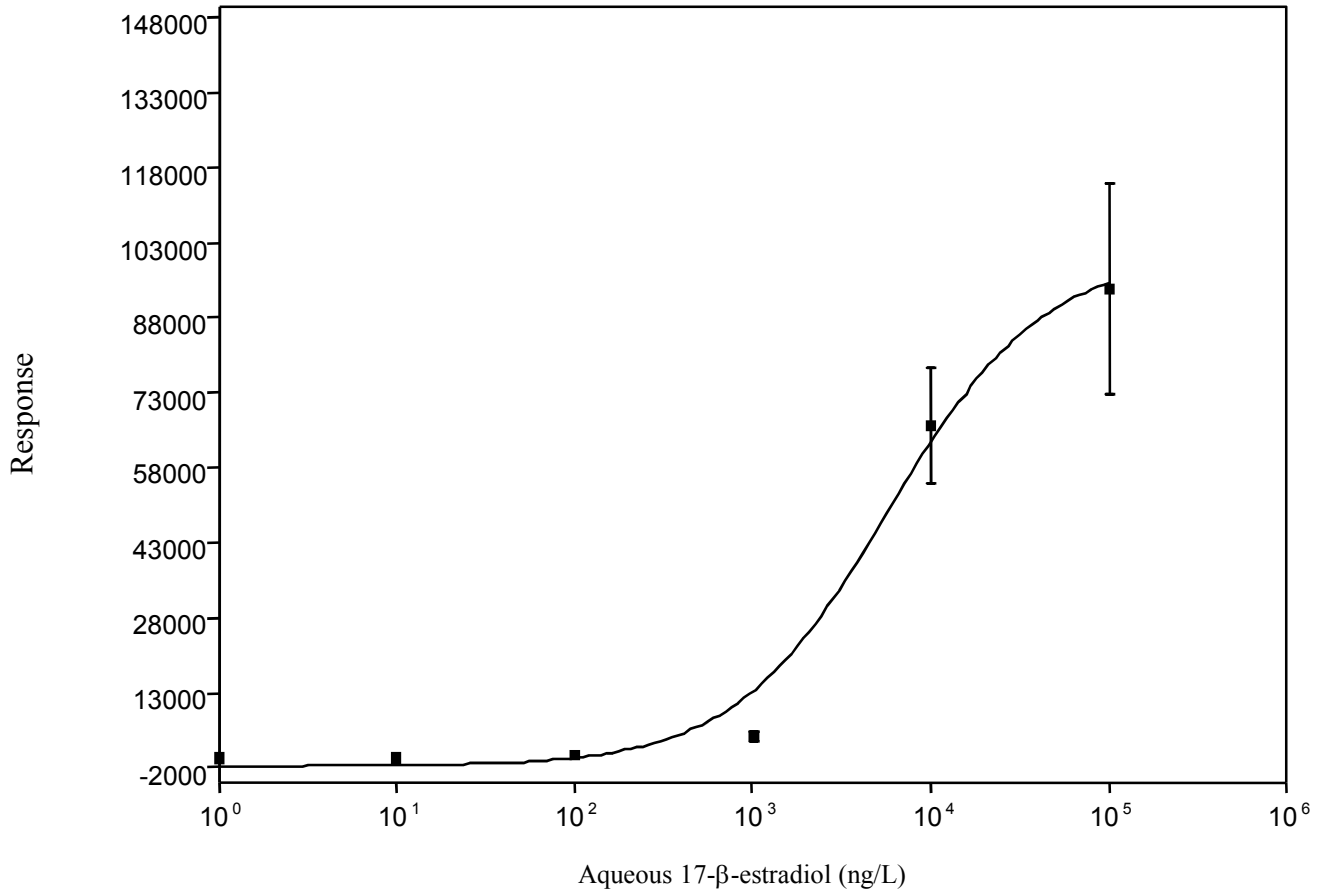
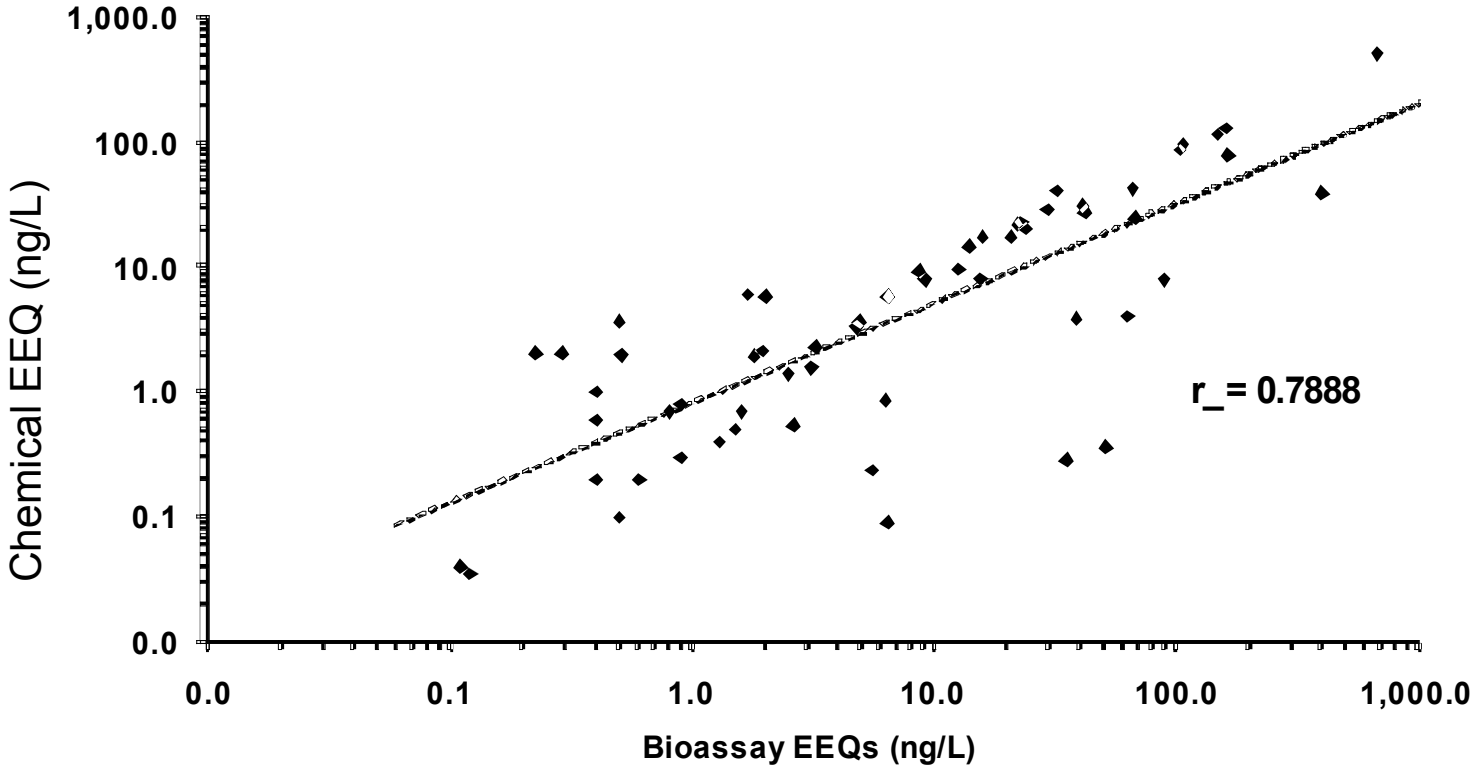
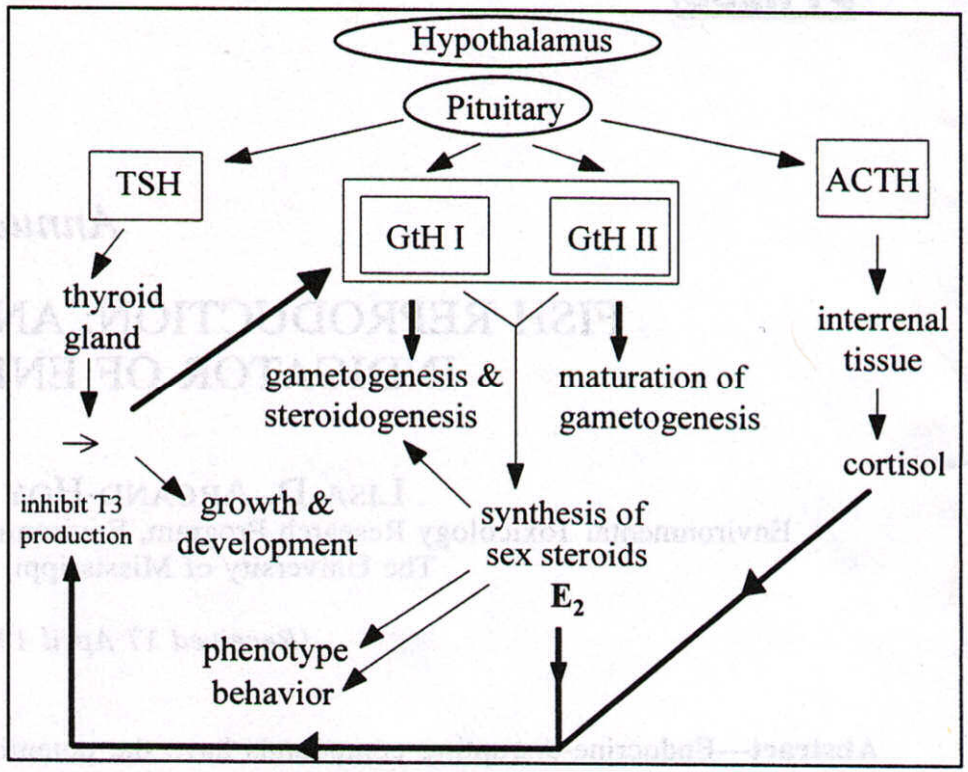


Figure II. Relationship between *in vitro* bioassay EEQs and chemically estimated EEQs in various studies (30, 33, 36, 38, 39, 64-67).

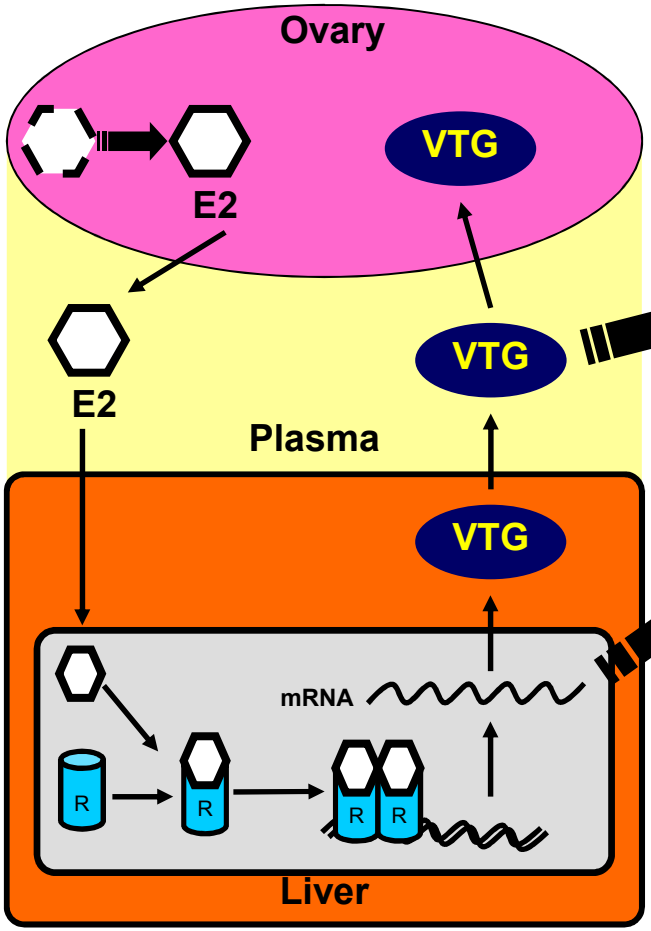




Arcand-Hoy and Benson 1998

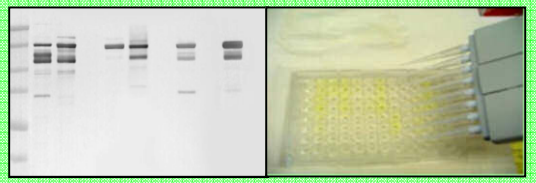
Material & Methods: Estrogenicity

VTG induction as a tool to evaluate estrogenic exposure in fish



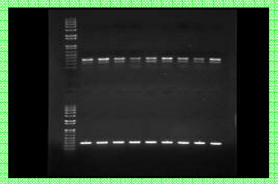
Determination of VTG

VTG protein detection



Western Blot ELISA

VTG mRNA detection

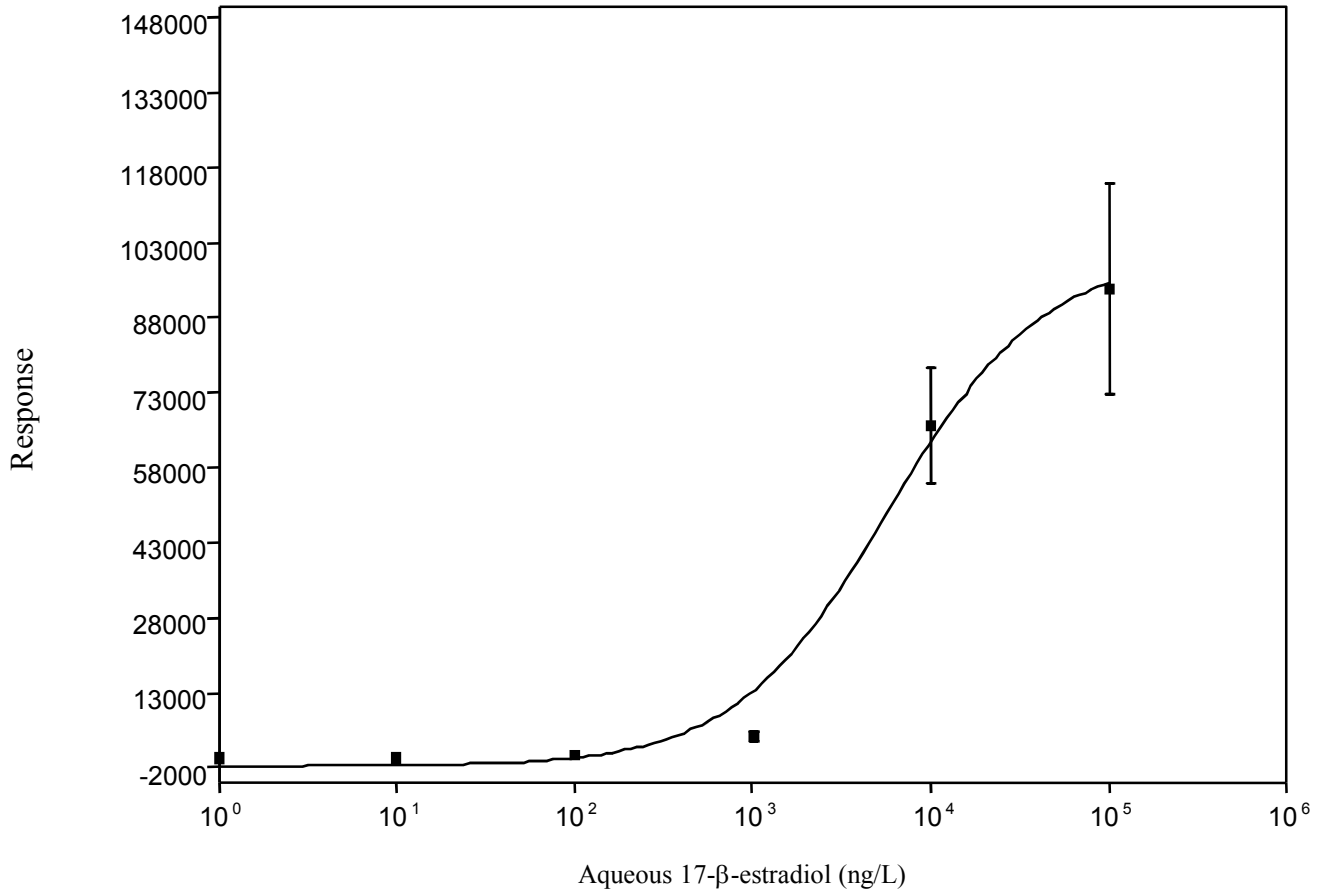


cDNA PCR

VTG induction in males:

Biomarker of exposure to ESTROGENIC COMPOUNDS

Example of EEQ Concentration-Response Curve



Collapse of a fish population after exposure to a synthetic estrogen

Karen A. Kidd*†, Paul J. Blanchfield*, Kenneth H. Mills*, Vince P. Palace*, Robert E. Evans*, James M. Lazorchak‡, and Robert W. Flick‡

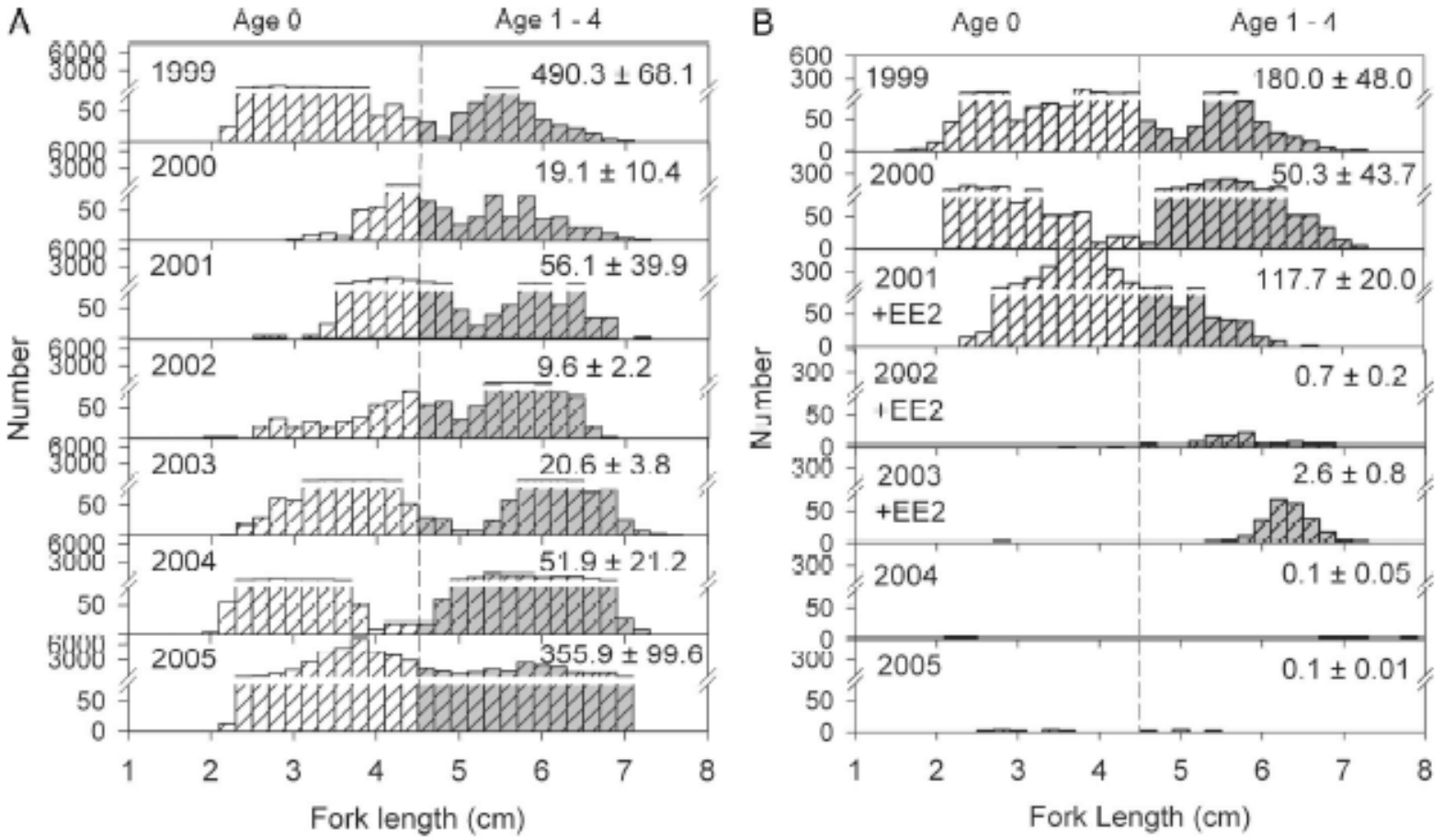


Fig. 3. Length frequency distributions of fathead minnow captured in trap nets in reference Lake 442 (*A*) and Lake 260 (*B*) (amended with 5–6 ng·L^{-1} of EE2 in 2001–2003) during the fall of 1999–2005. Distributions for each fall have been standardized to 100 trap-net days. Mean ± SE daily trap-net CPUE data for adults and juveniles for the fall catches are shown in the panels.

What about SF Delta?

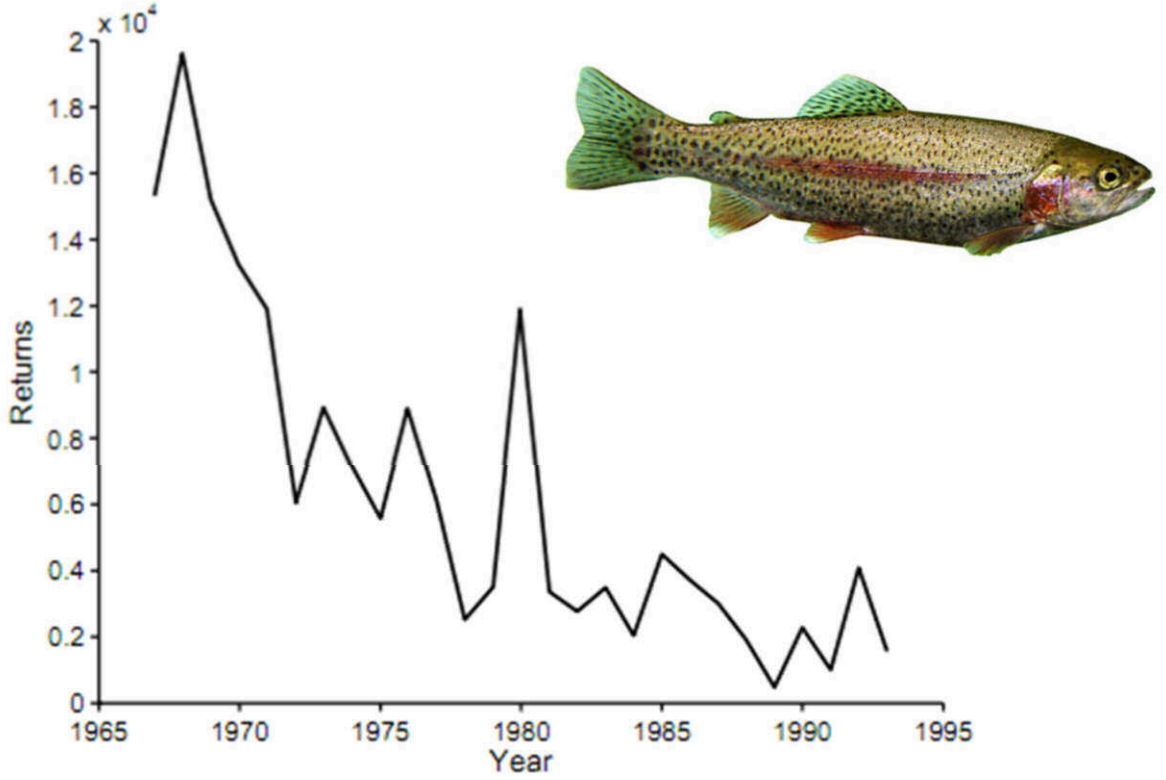
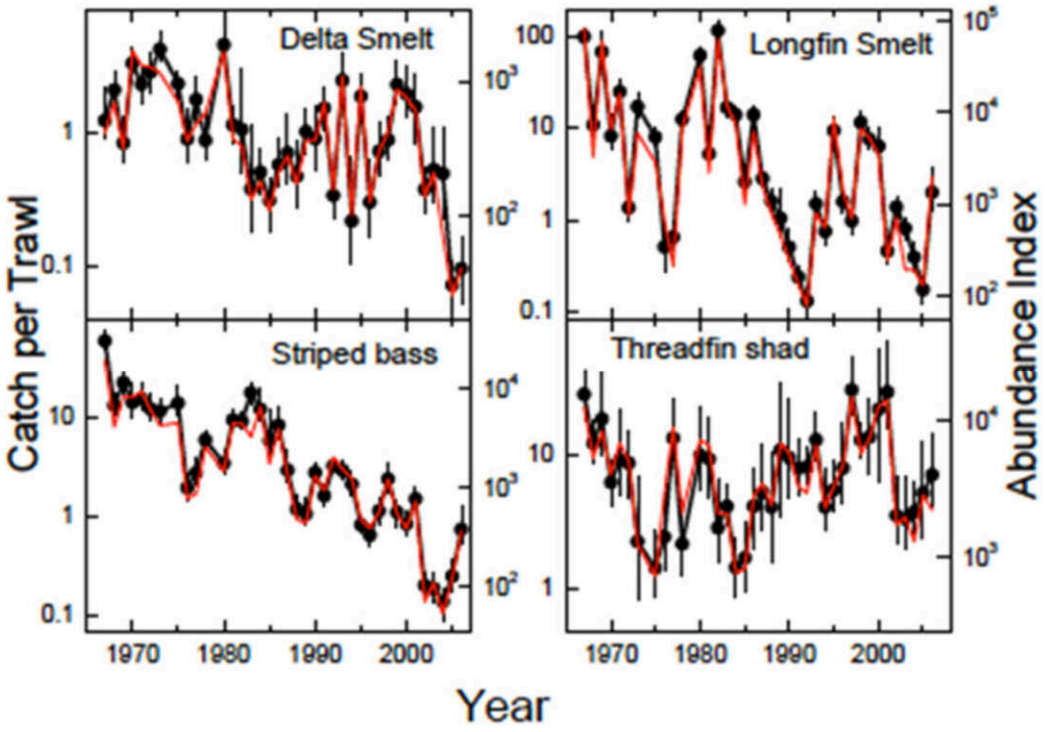


Figure 191. Returns of steelhead passing the Red Bluff Diversion Dam fish ladders, 1966–1994. These fish include hatchery fish from Coleman National Fish Hatchery.

Good et. al., 2005

Pelagic Organism Decline



Sommers et al. 2009

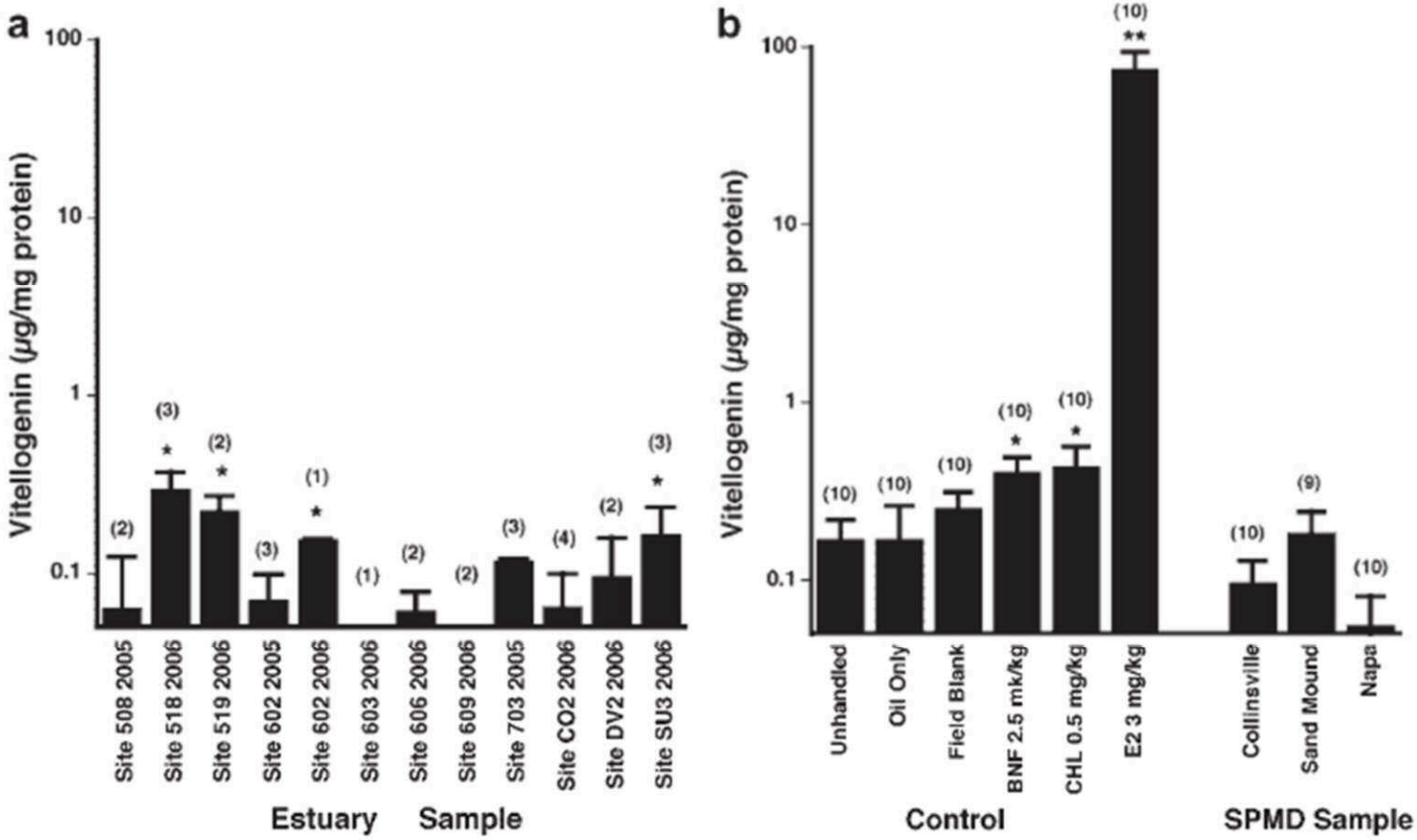
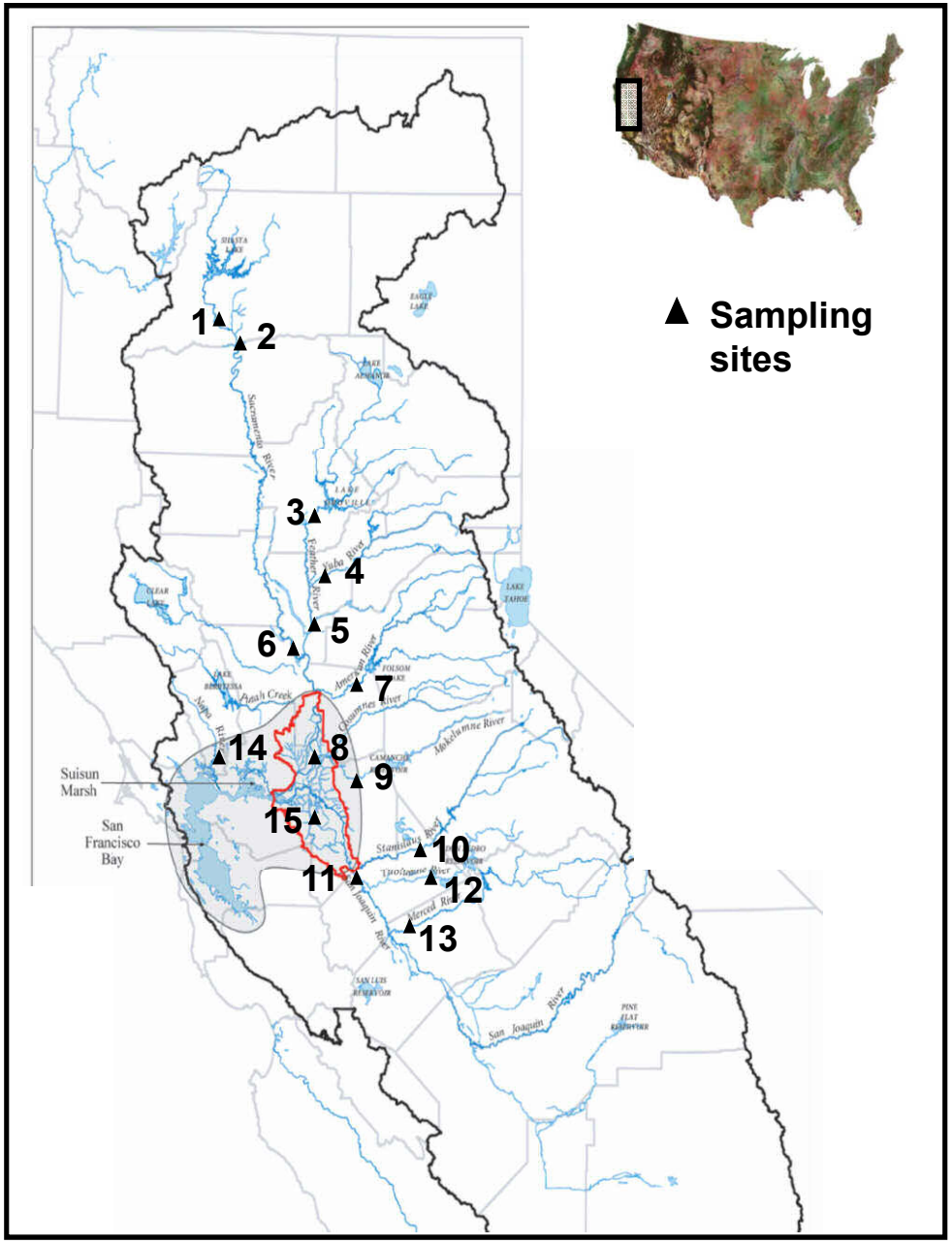


Fig. 4. Vitellogenin levels in striped bass exposed to contaminants present in the San Francisco Estuary (CA, USA) through field exposures (**a**), or through laboratory bioassays with injection of control and semipermeable membrane device (SPMD) extracts (**b**). Mean ± standard error of the mean for the number of individuals shown in parentheses. $^{*}p < 0.05$; $^{**}p < 0.01$ according to Tukey-Kramer multiple range test. Positive controls include beta-naphthoflavone (BNF), chlorpyrifos (CHL), and estradiol-17 beta (E2).

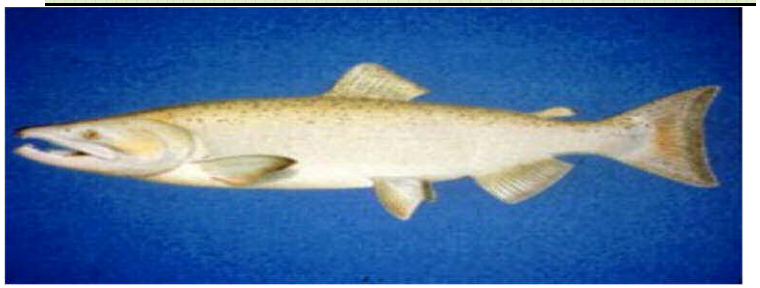
Spearow et al. 2011

Material & Methods: Sampling sites



▲ Sampling sites

Site	Site name
1	Upper Sacramento River
2	Battle Creek
3	Upper Feather River
4	Yuba River
5	Lower Feather River
6	Lower Sacramento River
7	Lower American River
8	Sacramento River in Delta
9	Mokelumne River
10	Stanislaus River
11	San Joaquin River
12	Tuolumne River
13	Merced River
14	Napa River
15	Clifton Court Forebay

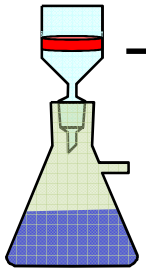


Material & Methods: Extracts & Exposures

Water extracts

Water (1 L)

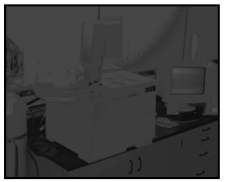
Solid-phase Extraction



C18 Disks

Elution
10 mL MetOH

Hormone determination (GC-MS)



Exposures *in vitro/in vivo*

Extract exposures

In vitro hepatocytes exposure

Primary Hepatocytes isolation and culture

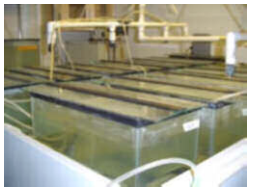
Extract added in the media (0.6% v/v)

Incubation
24 h

Total mRNA extract

VTG mRNA determination

In vivo whole fish exposure



Intraperitoneal extract injection (x2)

Incubation
7 days

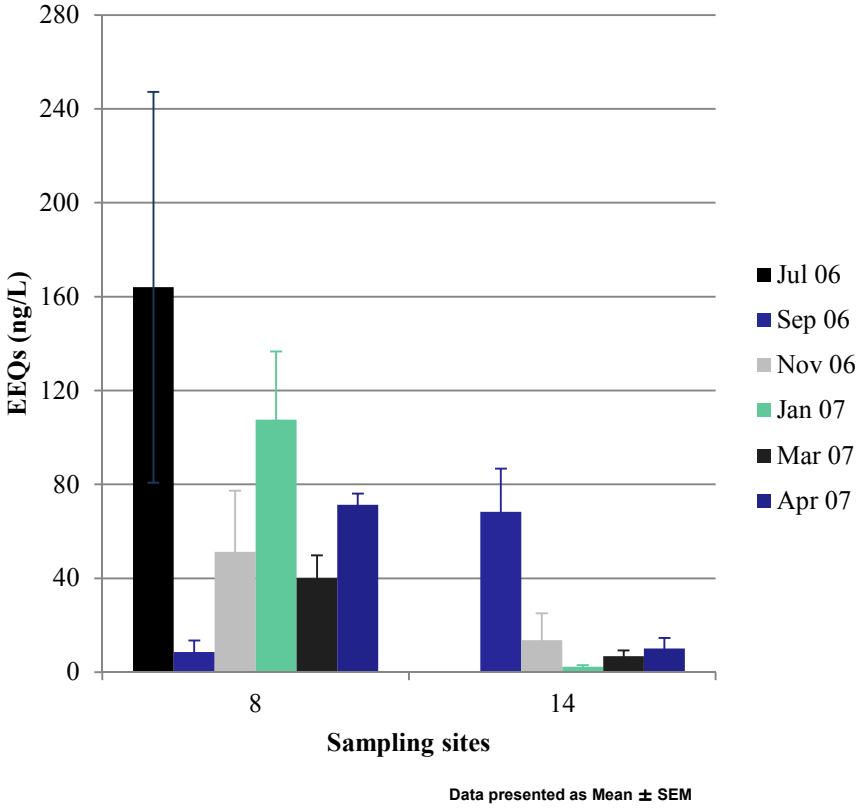
Plasma collection

VTG protein levels determination


Results: Water extracts

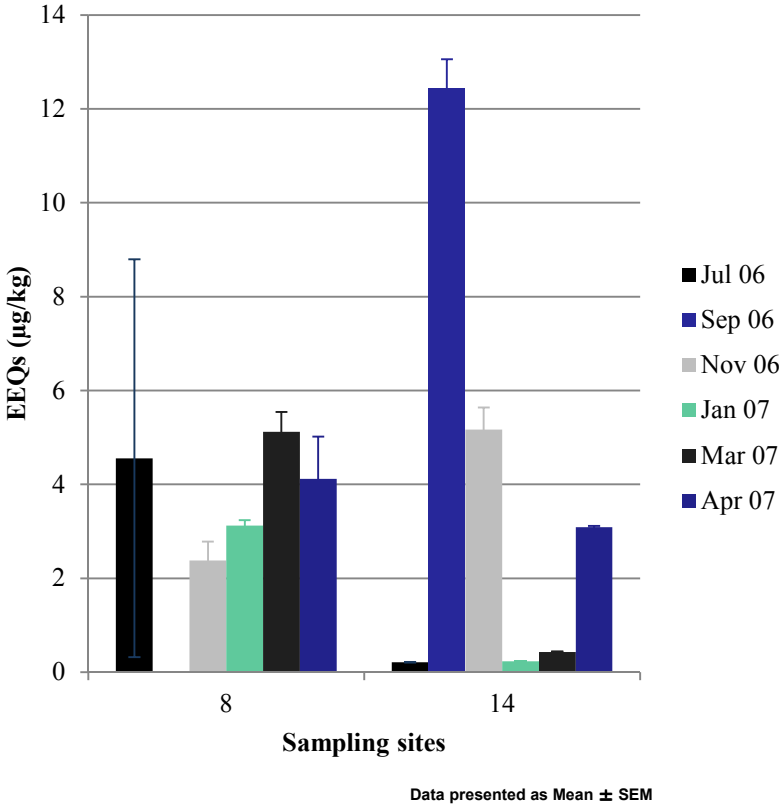
High Estrogenicity Areas (both methods)

Cells Exposure



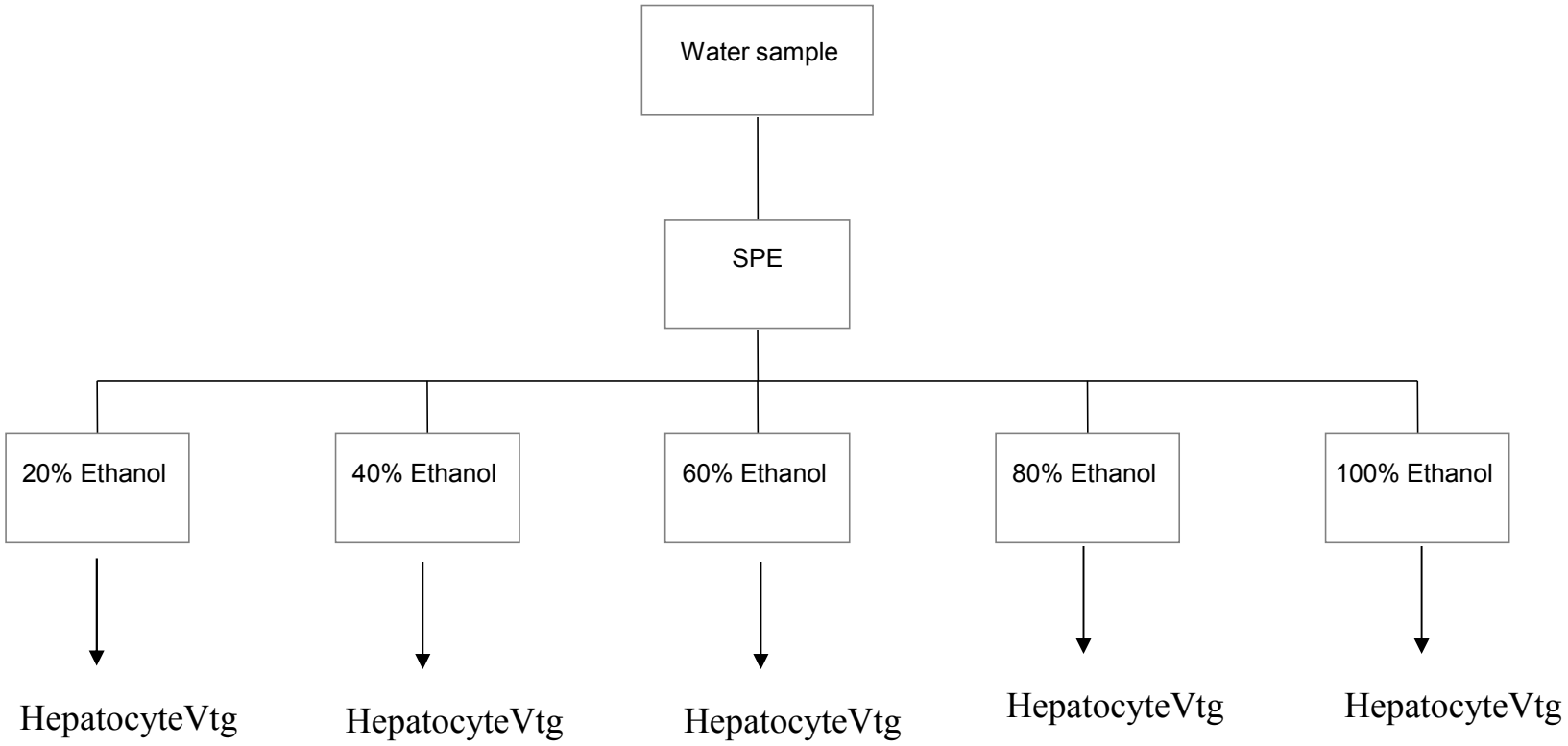
Data presented as Mean ± SEM

Whole Animal Exposure



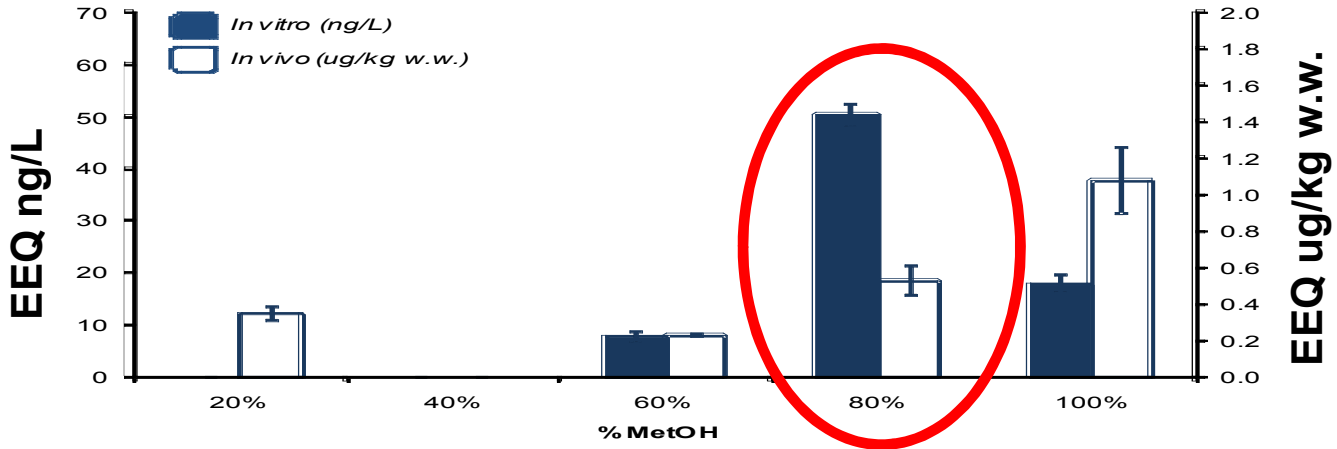
Data presented as Mean ± SEM

Toxicity Indentification Evaluation on Water Extracts from SF Bay Delta for the Determination of unknown estrogens.

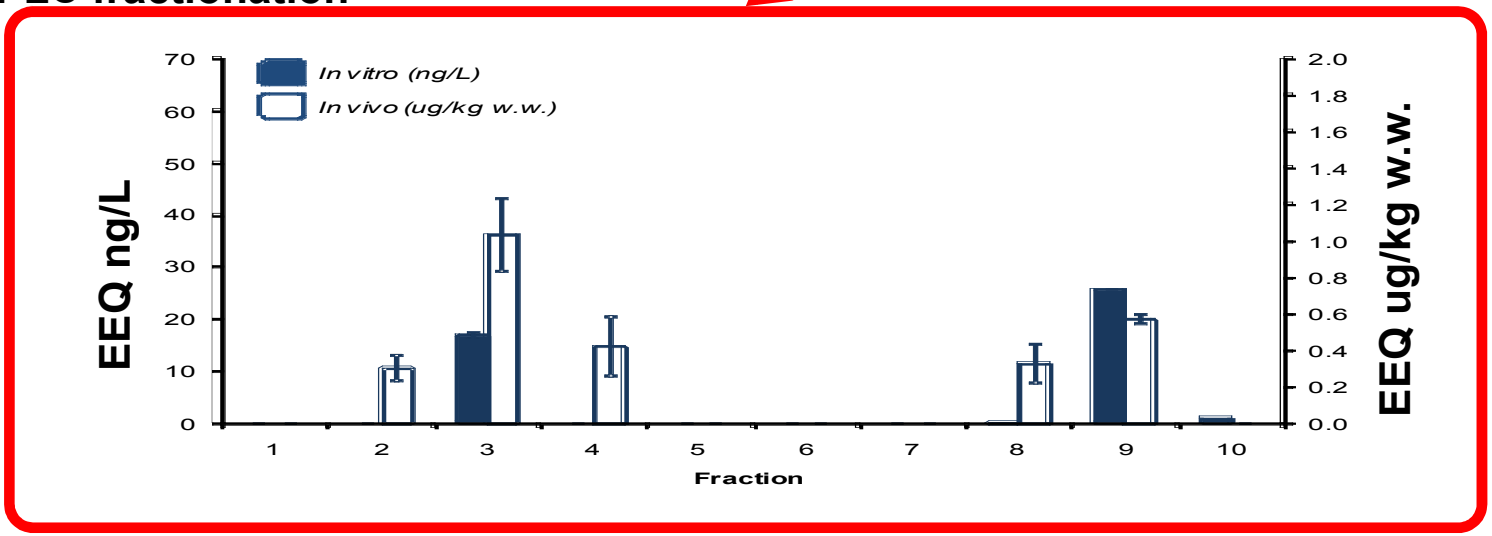


NAPA River Delta

SPE fractionation

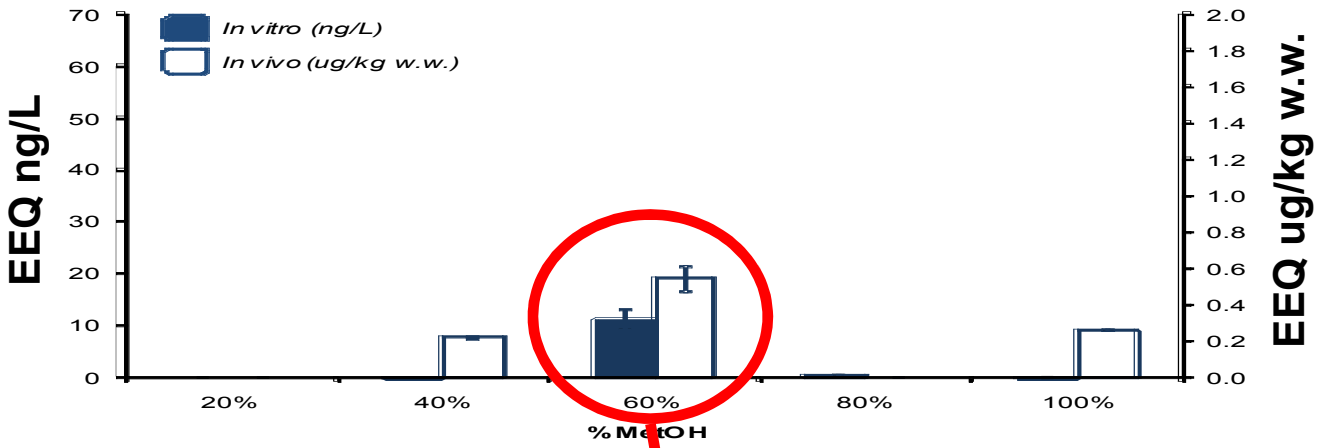


HPLC fractionation

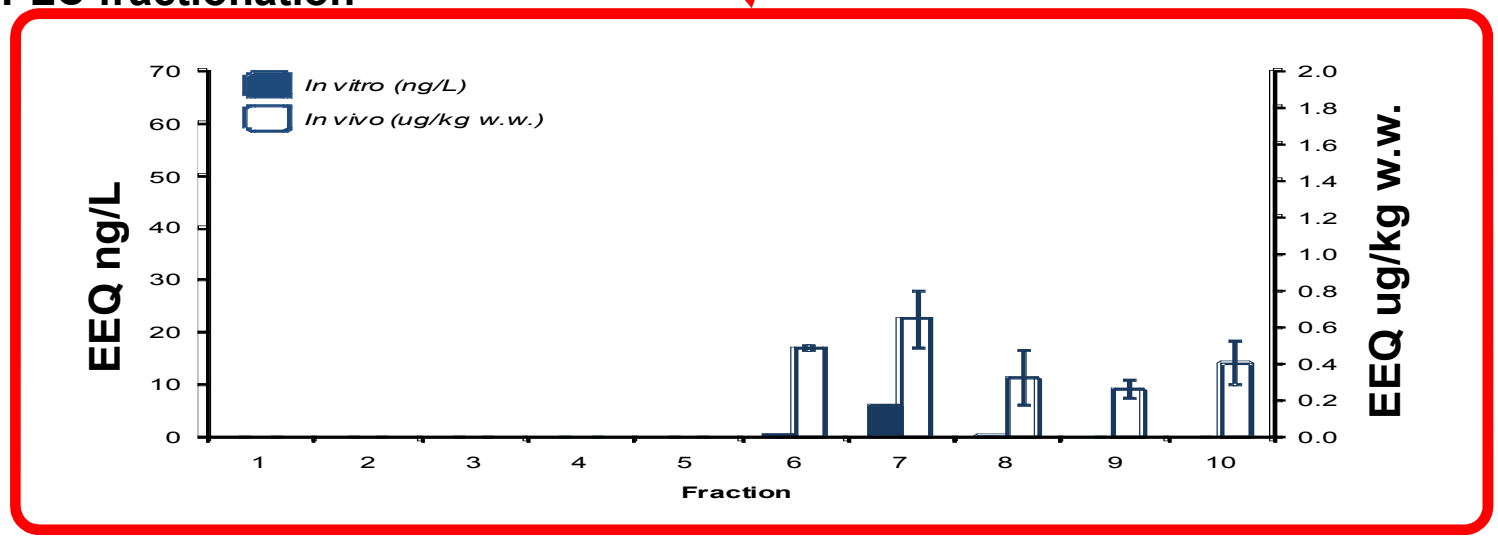


SACRAMENTO RIVER DELTA

SPE fractionation



HPLC fractionation



Measured Analytes for Bioactive Fractions

Phytoestrogens	Pharmaceuticals & Personal Care Products	Pesticides			Steroid Hormones	APEO/AP
Genistein	Sulfamethoxazole	Aldicarb	Promecarb	Secbumeton	17β-Estradiol	4-Octylphenol
Daidzein	Atenolol	Aldicarb Sulfone	Propham	Simazine	Estrone	OP1EO
Formononetin	Trimethoprim	Aldicarb sulfoxide	Siduron	Simetryn	Estriol	OP2EO
Biochanin A	Iopromide	Aminocarb	Swep	Terbuthylazine	Progesterone	4-Nonylphenol
Apigenin	Caffeine	Barbamate	Chlorpyrifos	Terbutryn	Testosterone	NP1EO
Naringenin	Fluoxetine	Baygon	Diazinon	Thiobencarb	Androstendione	NP2EO
Coumestrol	Meprobamate	Captan	Imidacloprid	DEET	Ethynylestradiol	
Chrysin	Dilantin	Carbaryl	Myclobutanil	Permethrin		
Matairesinol	Carbamazepine	Carbofuran	Oryzalin	Cypermethrin		
Equol	Diazepam	Carbofuran phenol-3-ketone	Oxyfluorfen	Deltamethrin		
Glycitein	Atorvastatin	Chlorpropham	Tebuconazole	Cyfluthrin		
	Benzophenone	Dioxacarb	Ametryn	Bifenthrin		
	Primidone	Diuron	Atraton	Triclopyr		
	TCPP	Fenuron	Atrazine	2,4-D		
	TCEP	Fluometuron	Cyanazine			
	Gemfibrozil	3-Hydroxycarbofuran	Deisopropyl-atrazine			
	Bisphenol A	Linuron	Desethyl-atrazine			
	Diclofenac	Methiocarb	Desmetryn			
	Naproxen	Methomyl	Dipropetryn			
	Triclosan	Monuron	2-Hydroxyatrazine			
	BHA	Neburon	Molinate			
	Musk ketone	Oxamyl	Prometon			
	Ibuprofen	Propazine	Prometryn			

Experimental groups and Doses

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30567!	!	!	!	!	!	!	!	!	!	!
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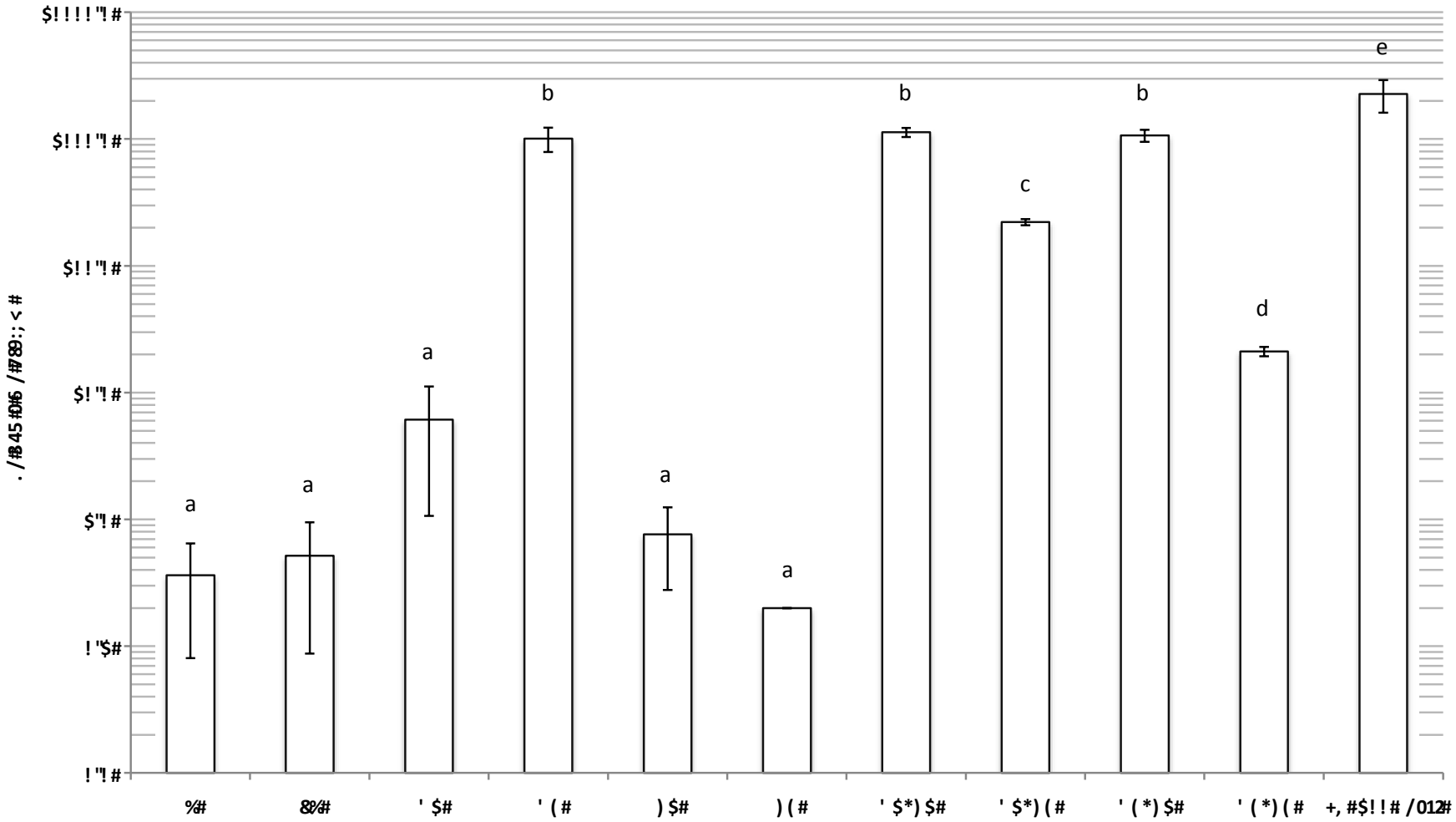
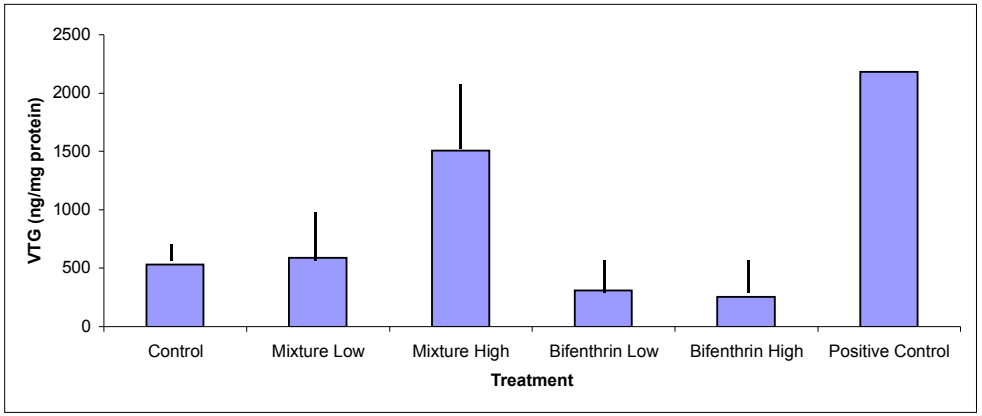
In vivo ESTROGENICITY of Pesticide APE Mixtures

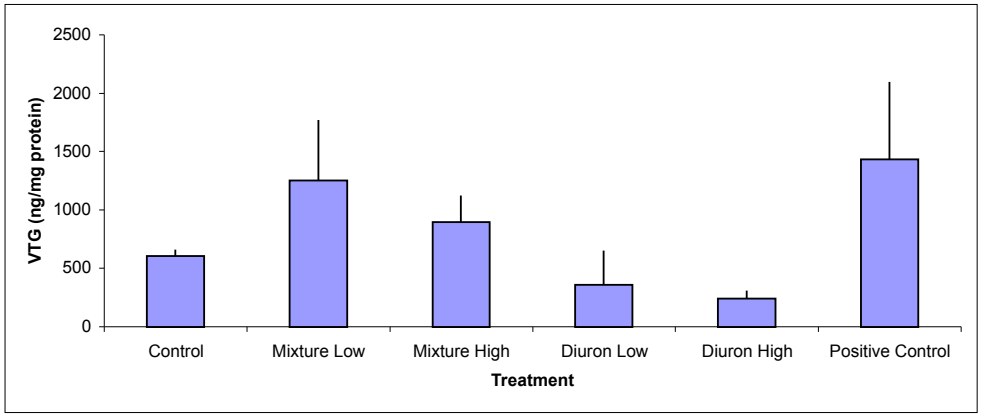


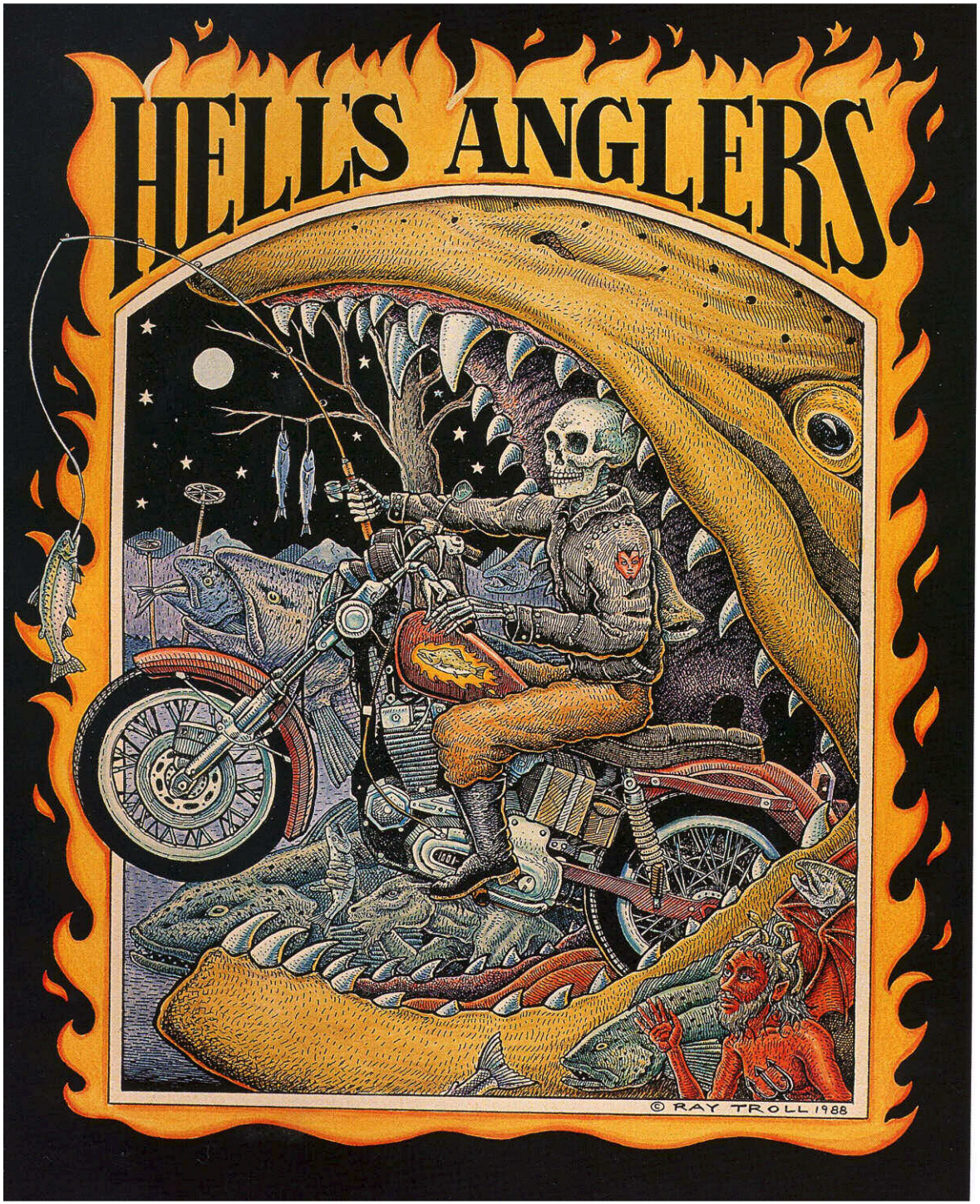
Data presented as MEAN ± SD. Different letter means significant differences (One-Way ANOVA, Tukey's Test, $p<0.05$)

Effects of Bifenthrin and APE mixture on Plasma Vtg in male Medaka



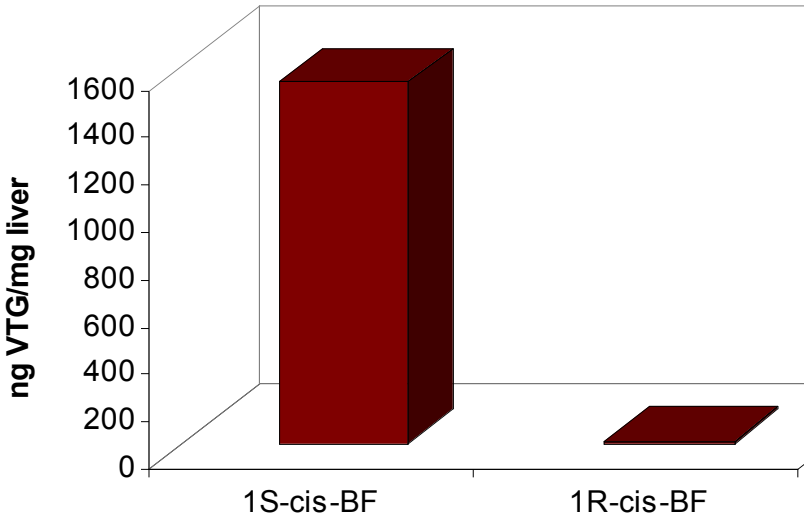
Effects of Diuron and APE mixture on Plasma Vtg in male Medaka

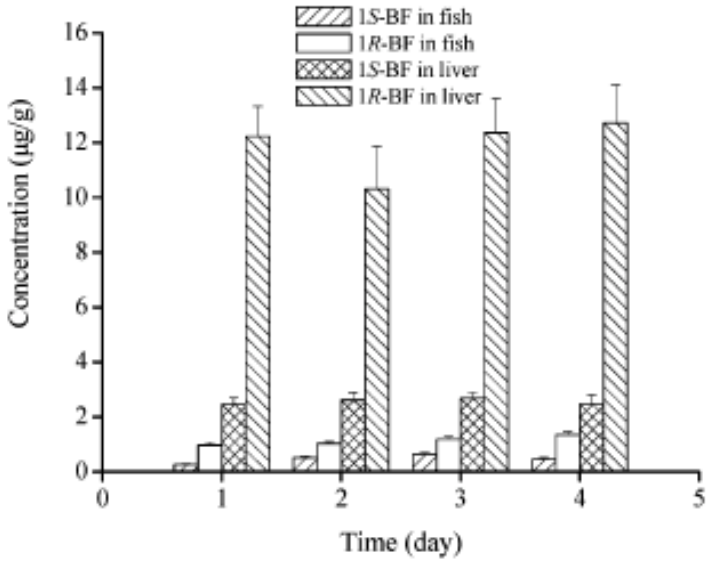



HELL'S ANGLERS
©RAY TROLL 1988

ESTROGENIC ACTIVITY AND PYRETHROIDS

1S-cis-BF is 123 times more active than 1R-cis-BF

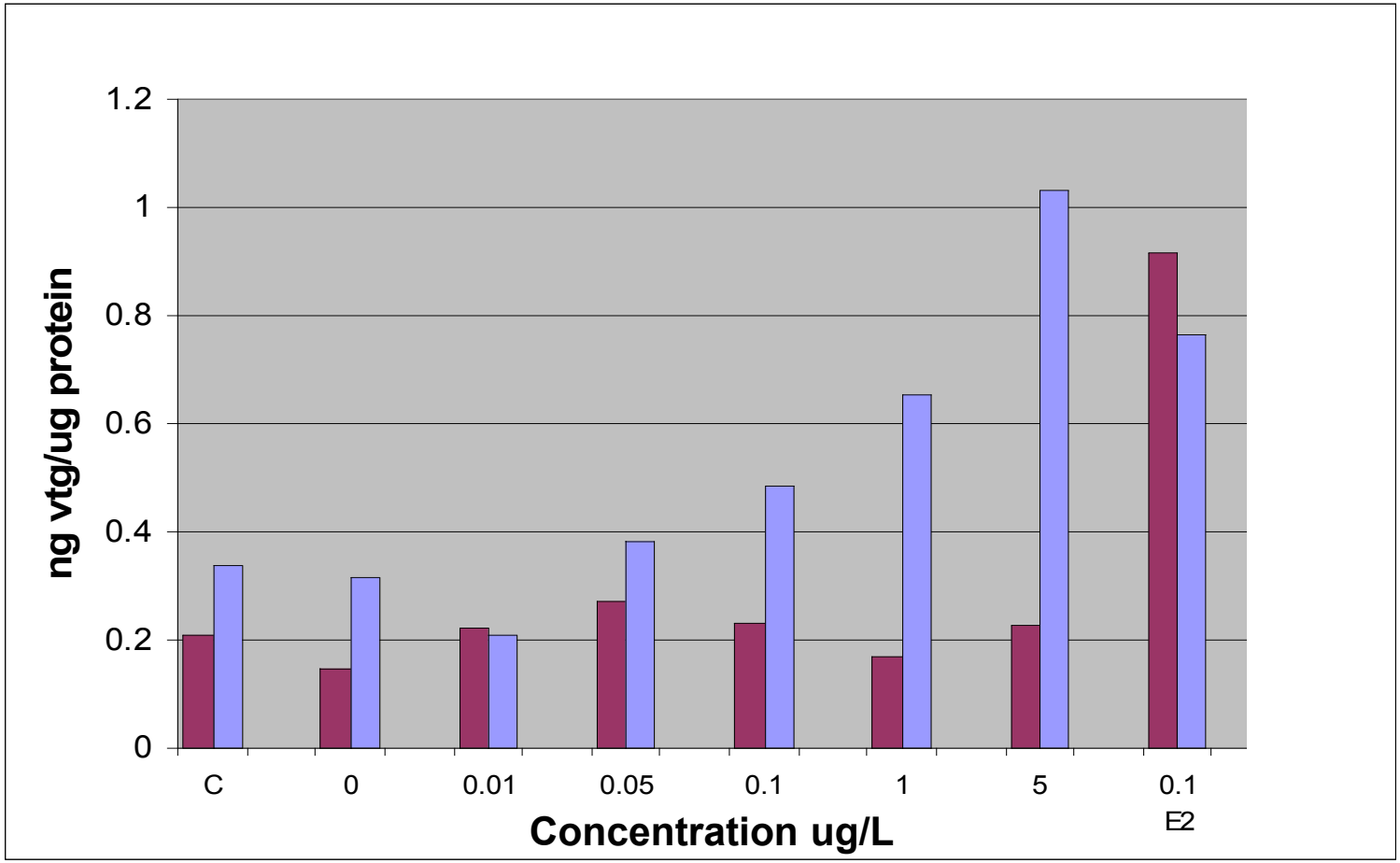




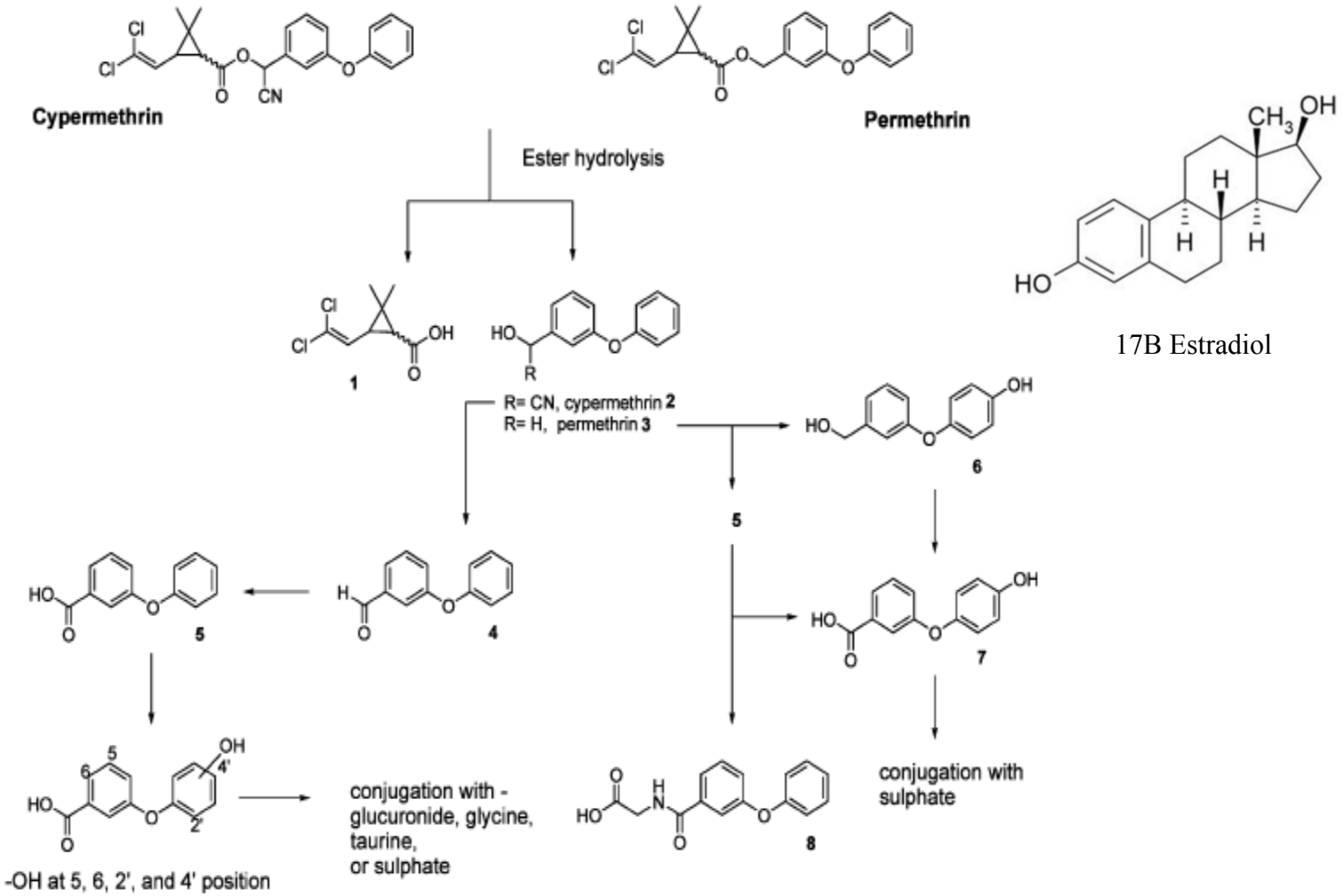
Enantioselectivity in in vivo vitellogenin induction in adult male Japanese medaka (ELISA Assay)

Wang et al., 2007, EST, 41: 6124-6128

Vitellogenin induction in juvenile medaka following exposure to cypermethrin (Red) and permethrin (Blue):

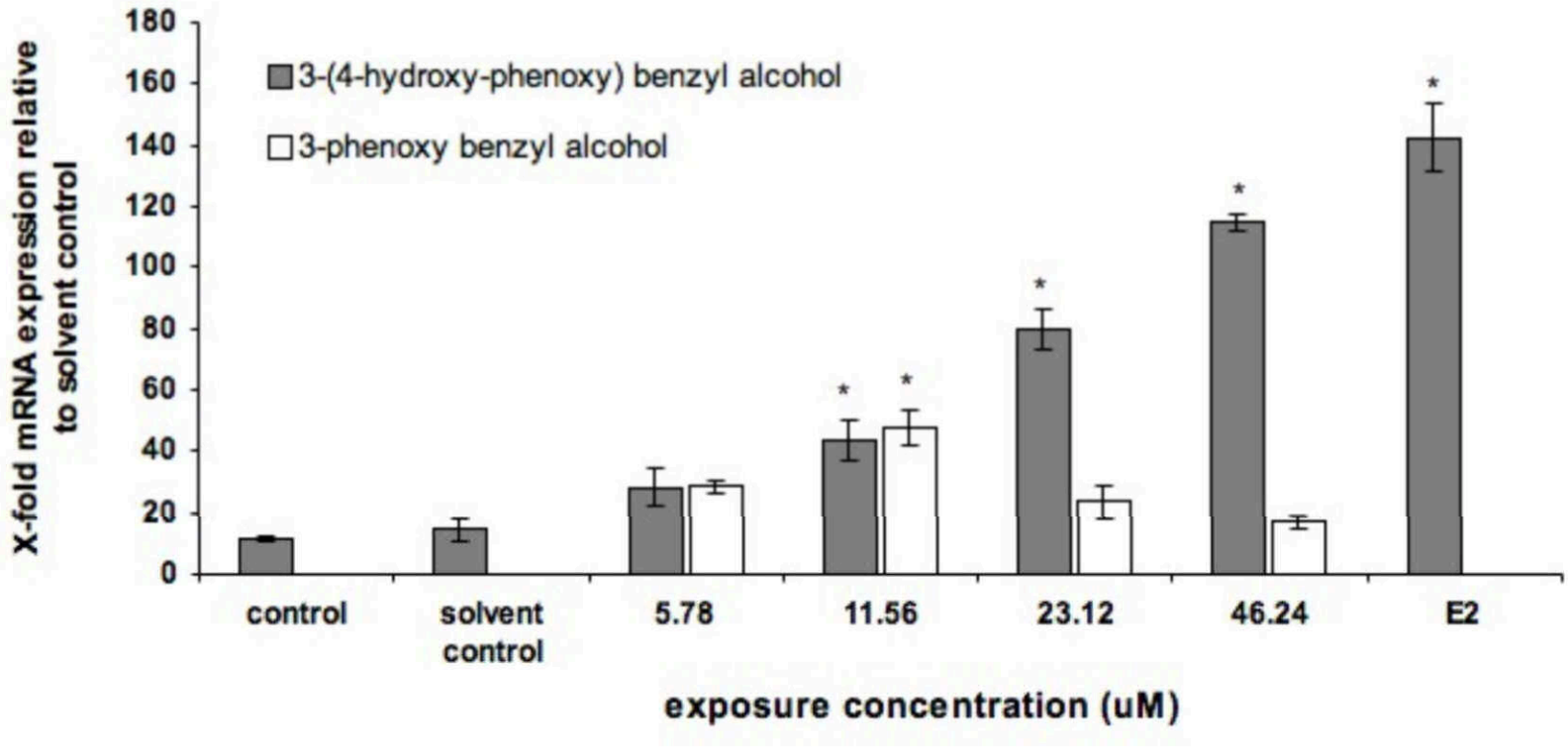


ESTROGENIC ACTIVITY AND PYRETHROID BIOTRANSFORMATION



(McCarthy et al., 2006, J. Environ. Monit., 8: 197-202)

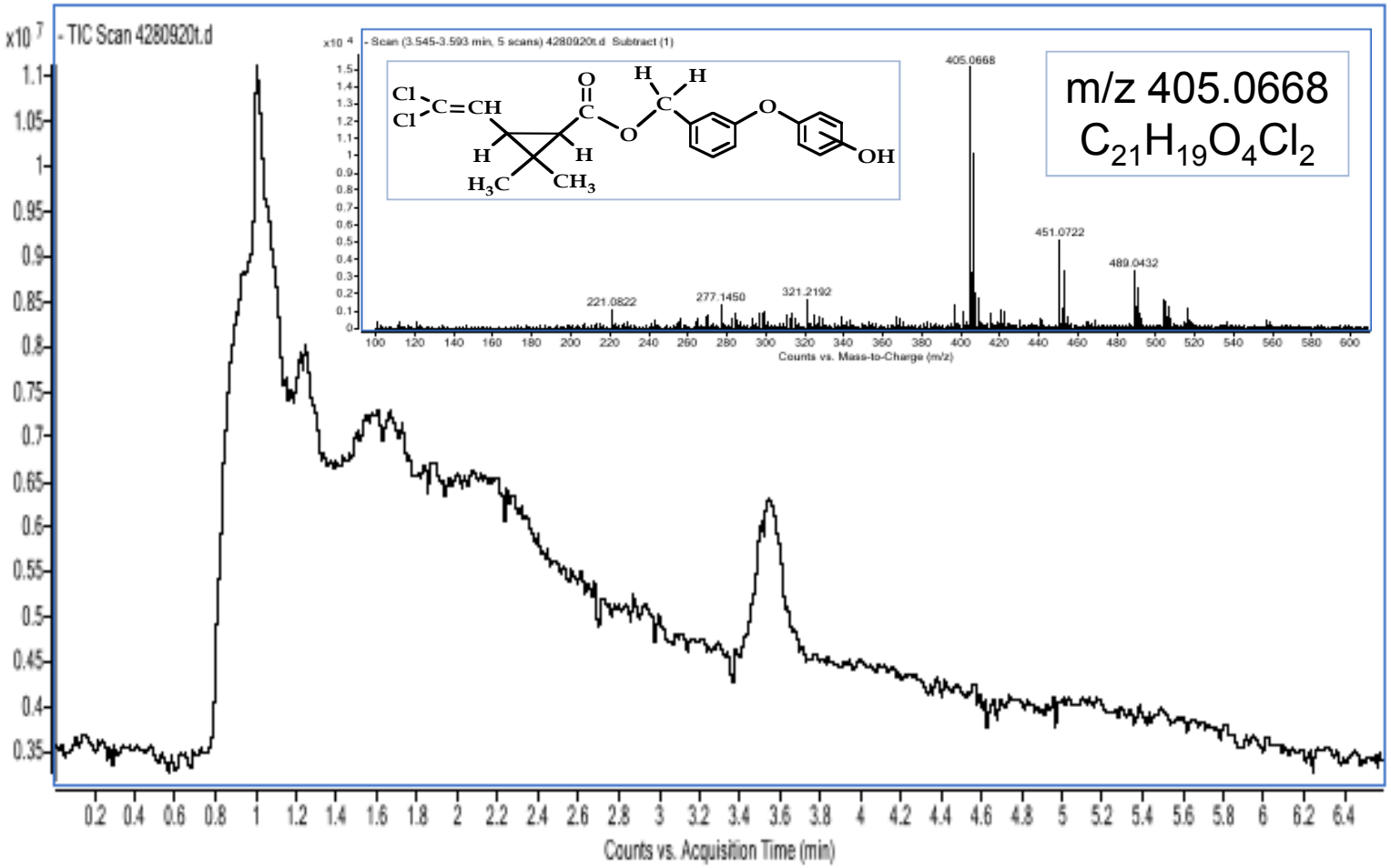
BIOACTIVATION TO ESTROGENIC METABOLITES



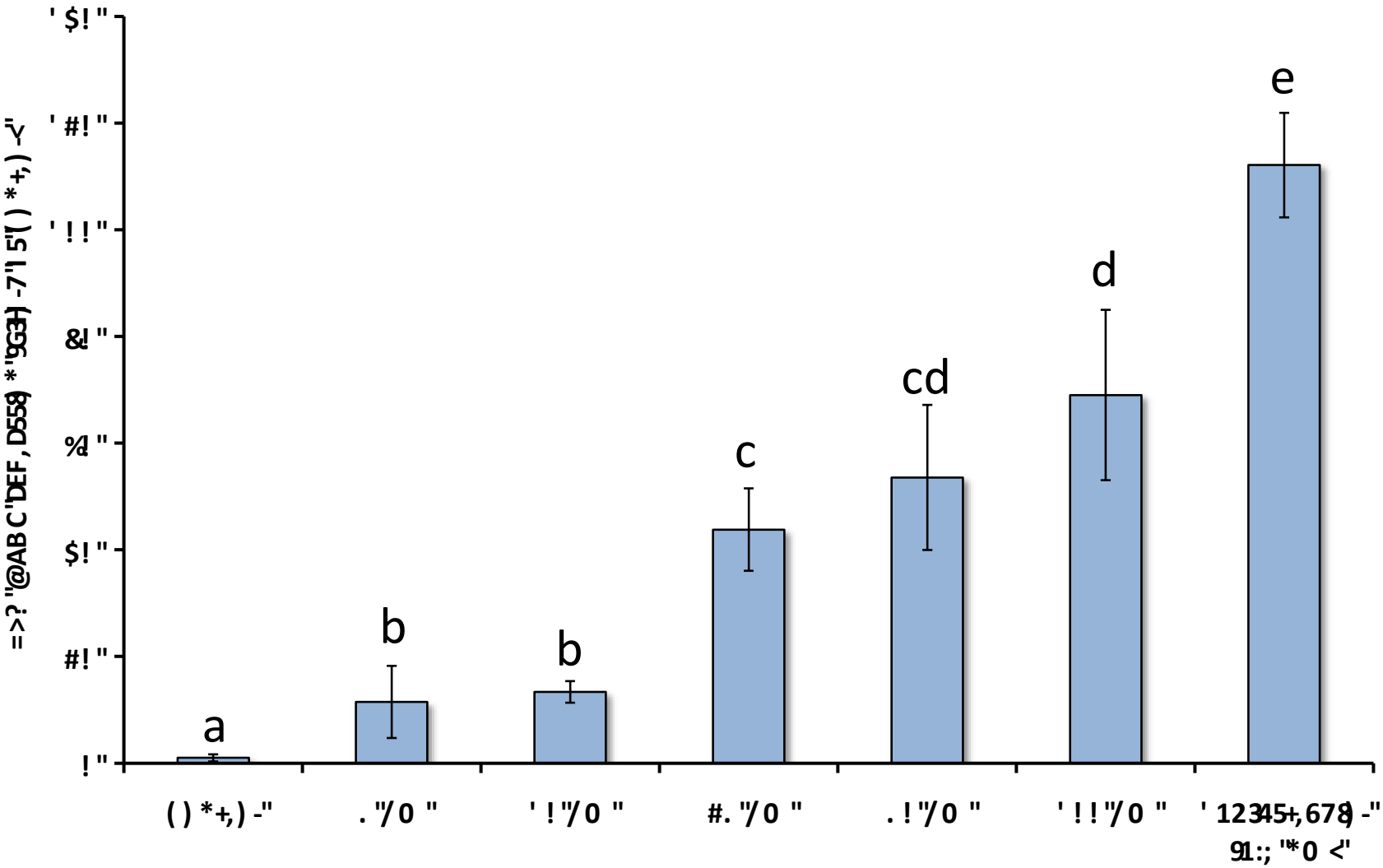
Values indicate mean ± SEM. Positive control (E2) concentration = 3.6 x 10^{-2} μM; solvent (acetone). * Indicate significant difference from solvent control ($p < 0.01$).

Nillos et al. 2010

Permethrin - Enantioselective Biotransformation

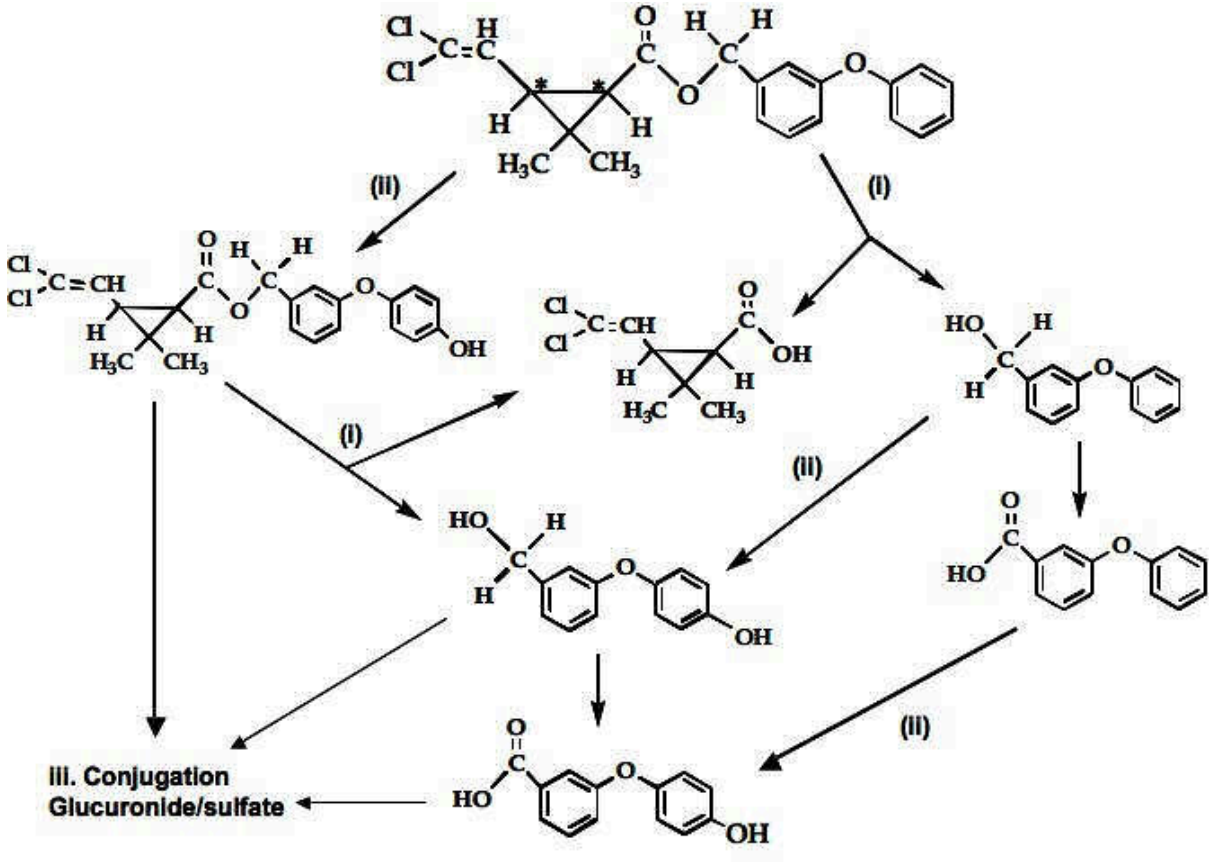


LC/MS TOF trace with accurate mass and molecular formula of major metabolite from *1S-cis*-permethrin metabolism in trout liver microsome. The most probable structure of metabolite is indicated. (Note: LC/MS trace and mass prectra of metabolite from *1S-trans*-permethrin metabolism is identical to the figure shown here).



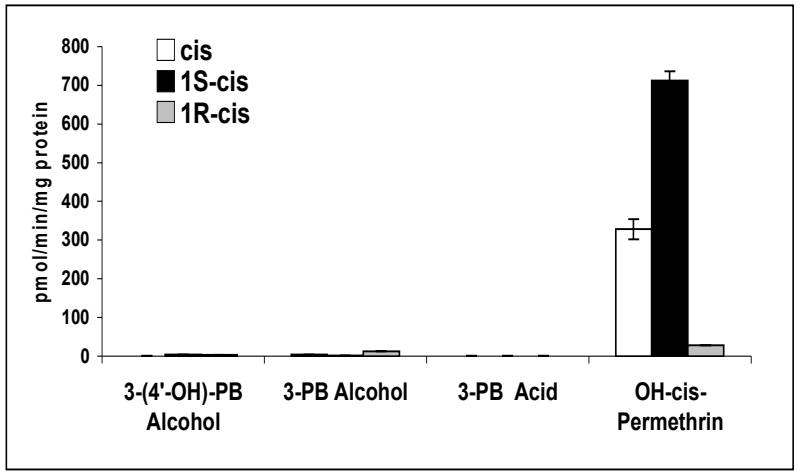
Data are presented as mean ± SD. 4 replicates per exposure (4 different wells of 1 million cells per well). Different letters mean significant differences (One-way ANOVA, Tukey's Test, $p<0.05$)

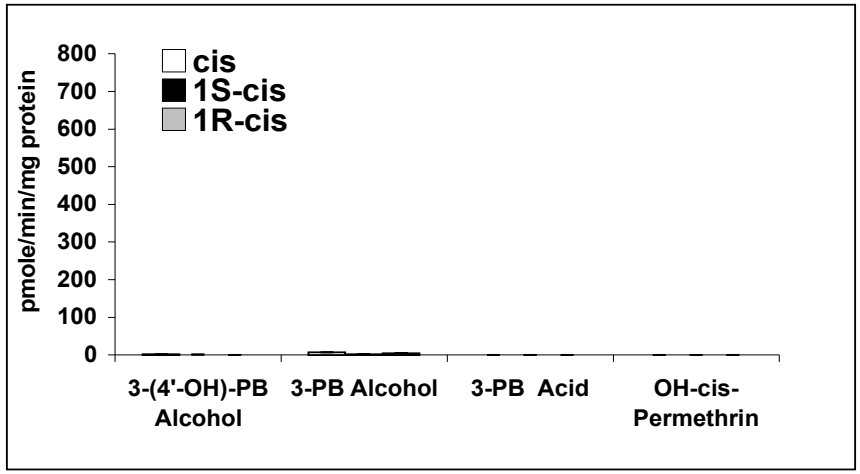
STEREOSELECTIVE BIOACTIVATION TO ESTROGENIC METABOLITES

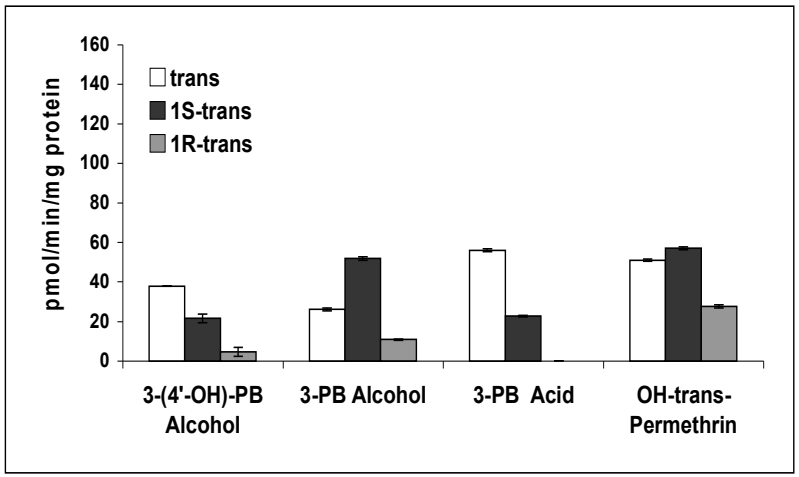


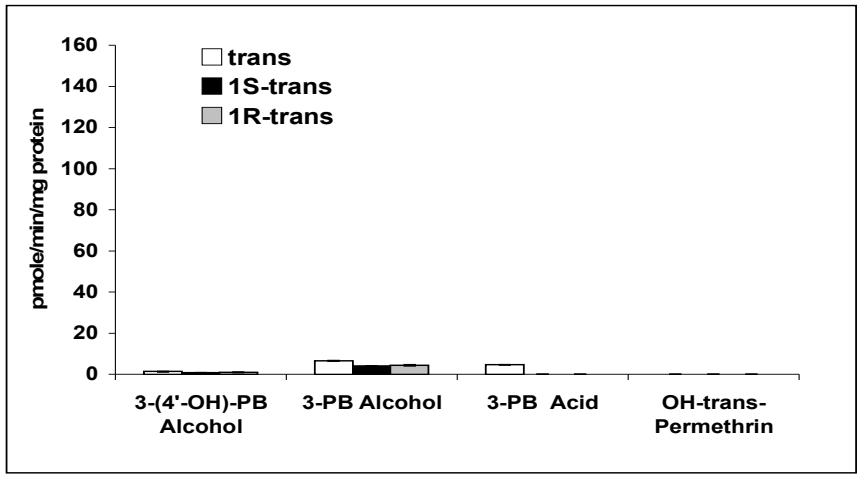
Biotransformation pathway of permethrin in fish

STEREOSELECTIVE BIOACTIVATION TO ESTROGENIC METABOLITES







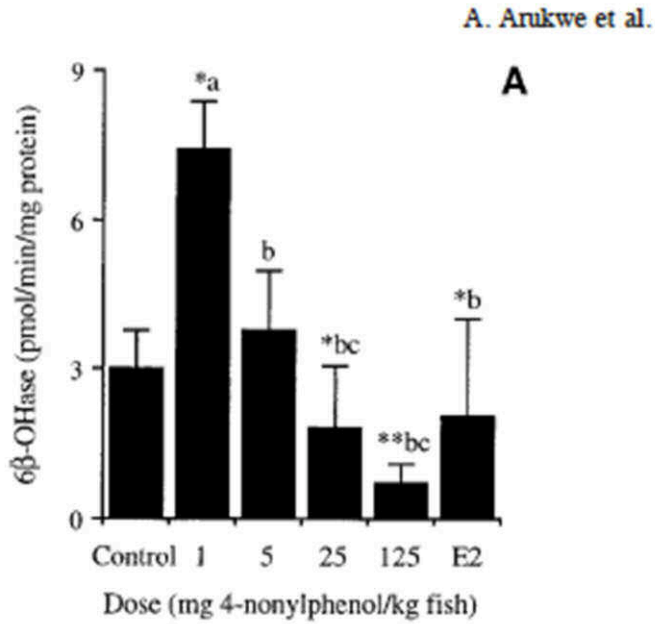


NO INHIBITOR

KETOCONAZOLE INHIBITION

The Big Picture

Alkylphenols → CYP3A Induction

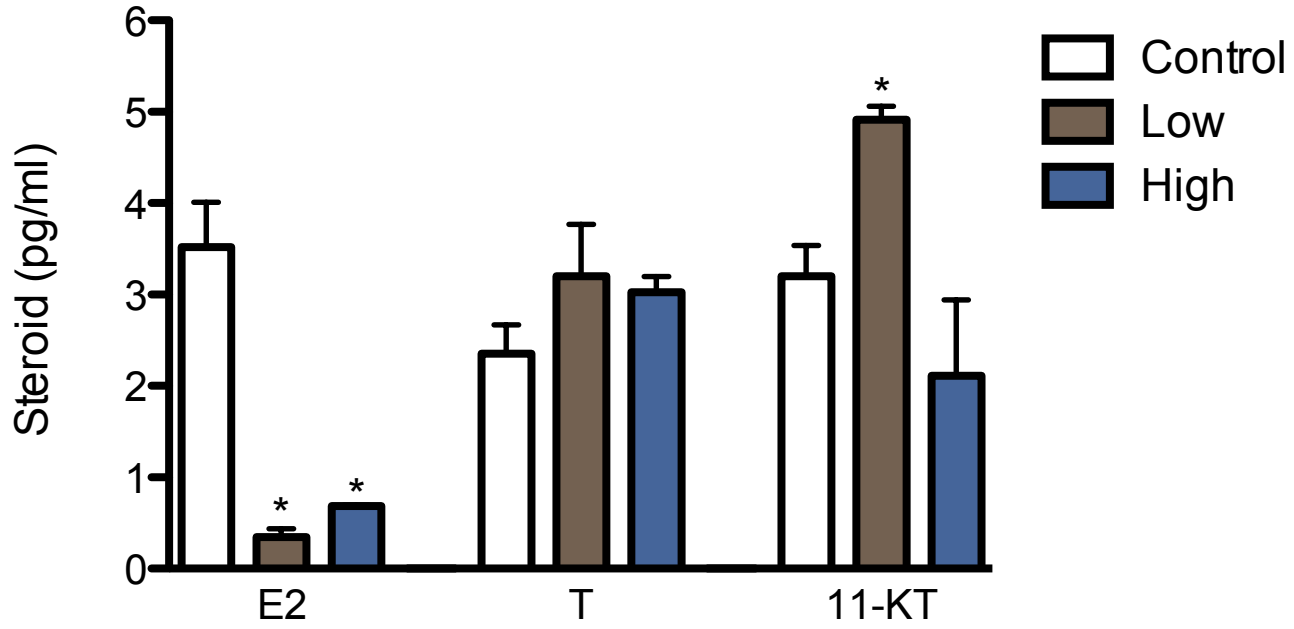


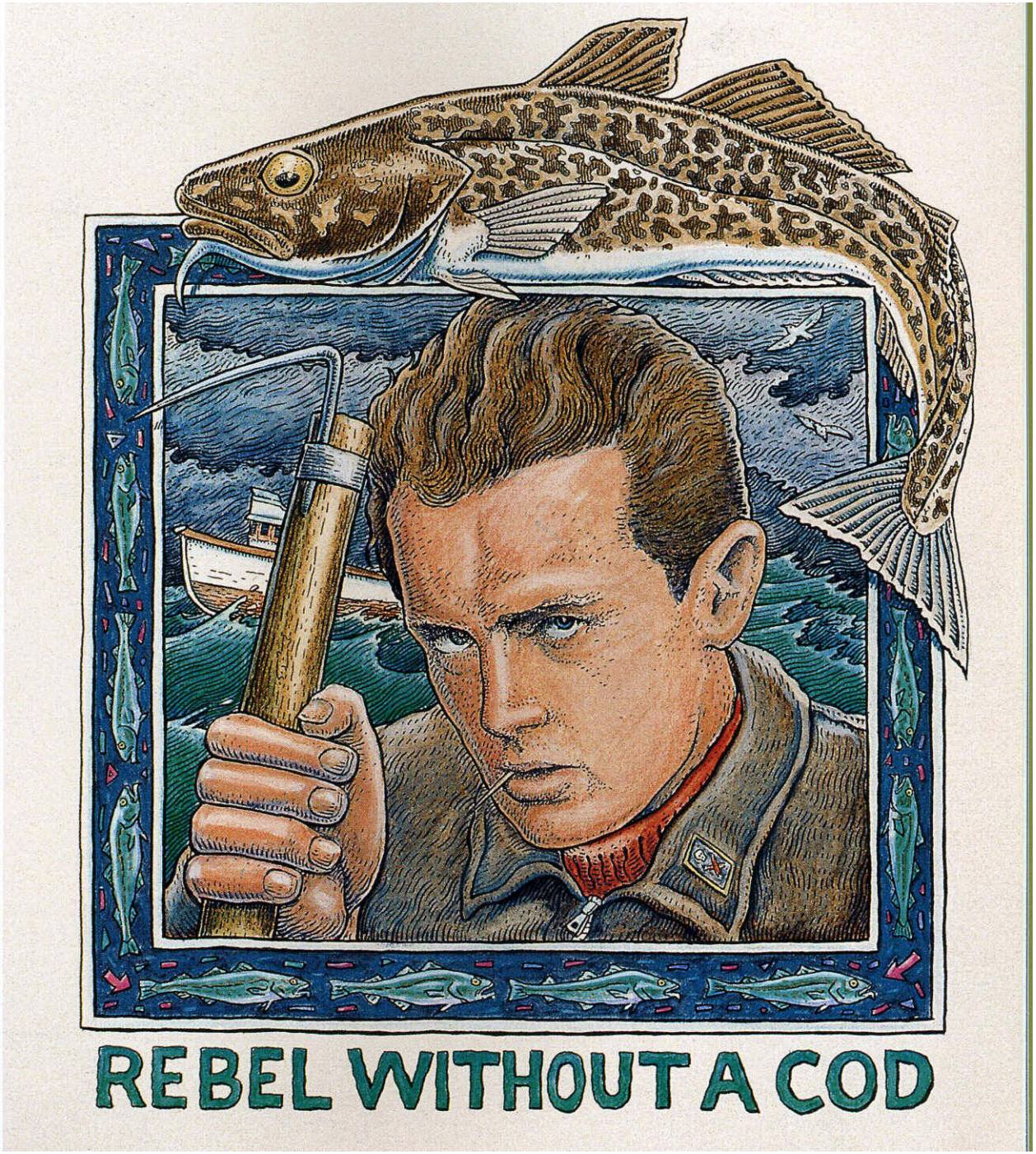
Pyrethroids → (CYP3A) Hydroxylated Metabolites → Vitellogenin Protein

\+

Estrogen Receptor (Transcription Factor)

Effects of 100 and 1500 ng/L Bifenthrin on plasma steroid concentrations in juvenile *O. mykiss* treated for 14 days.




REBEL WITHOUT A COD

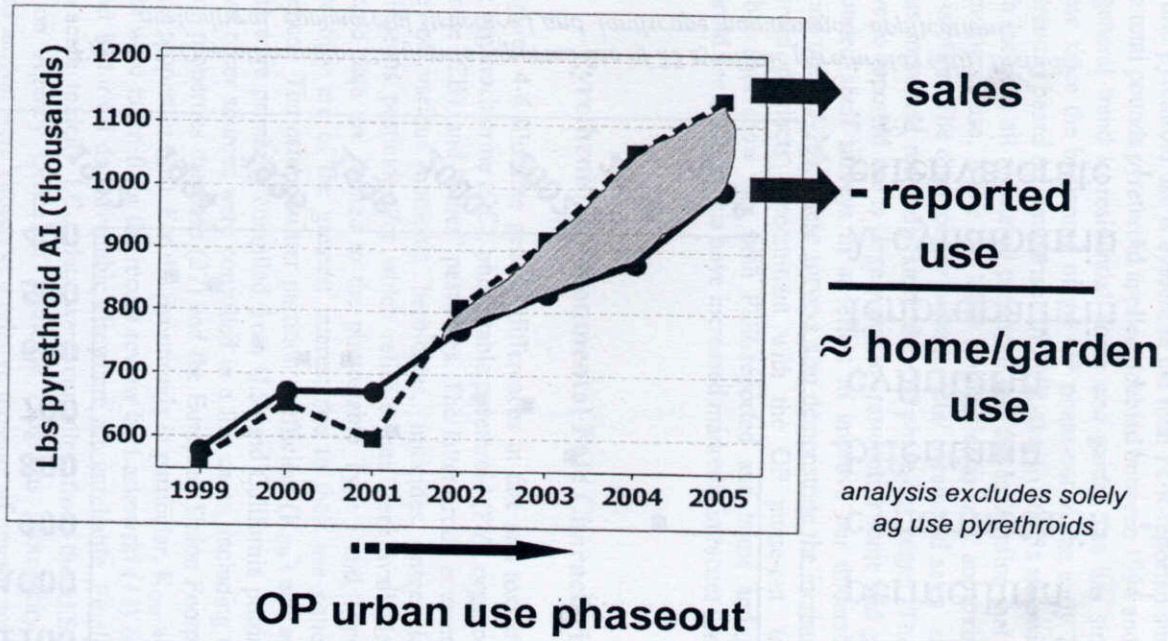
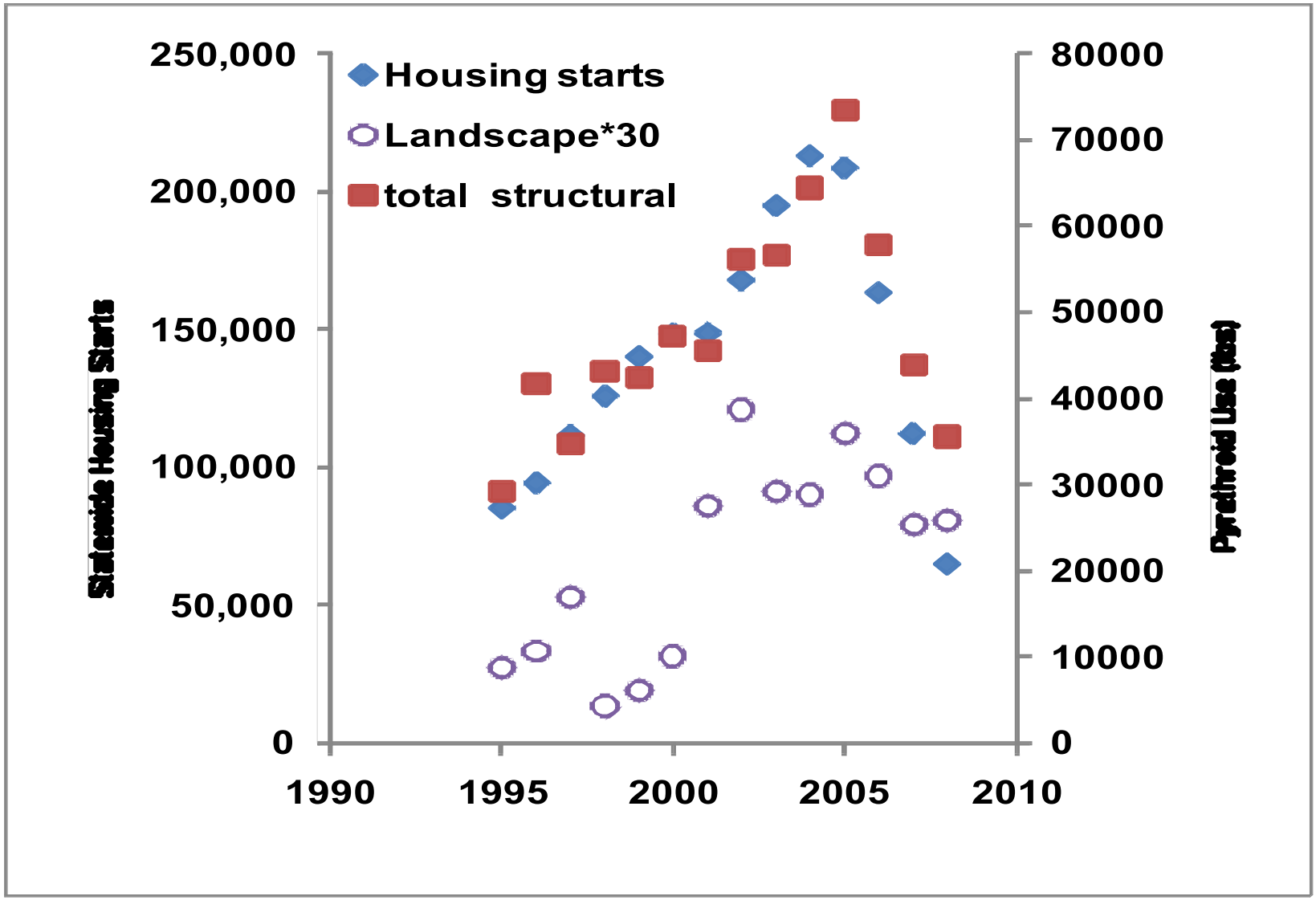


Figure 3. Approximate trend in residential home and garden pyrethroid use. Estimated by difference between sales(15) and reported use (16).

Urban Use Trends: Economic Factors



Returning Chinook Salmon for San Joaquin and Sacramento Rivers 2000-2010

2000	50,965	96,688	290,698	438,351	7,396	39,934	47,330	58,361	427,320	485,681
2001	61,318	75,296	453,323	589,937	7,391	27,303	34,694	68,709	555,922	624,631
2002	96,248	65,690	672,962	834,900	9,753	28,016	37,769	106,001	766,668	872,669
2003	118,097	89,229	362,161	569,487	8,666	12,839	21,505	126,763	464,229	590,992
2004	116,869	43,604	202,904	363,377	11,406	12,065	23,471	128,275	258,573	386,848
2005	187,427	57,012	172,457	416,896	5,984	14,813	20,797	193,411	244,282	437,693
2006	80,594	55,468	146,348	282,410	4,289	6,176	10,465	84,883	207,992	292,875
[2007]	21,682	17,061	54,559	93,302	1,130	1,709	2,839	22,812	73,329	96,141
[2008]	18,905	24,743	25,566	69,214	315	2,341	2,656	19,220	52,650	71,870
[2009]	20,904	5,827	22,842	49,573	1,799	1,757	3,556	22,703	30,426	53,129
[2010]	46,305	16,372	90,154	152,831	5,421	4,929	10,350	51,726	111,455	163,181

DFG Feb 2011

SF Delta Conclusions

- Chemistry alone inconclusive.
- Estrogenic activity was consistently observed in water extracts and whole water from surface waters.
- Bifenthrin, diuron, APE mixture detected in water samples from Napa site
- Reconstitution with individual compounds failed to show estrogenic effects when exposed to fish or cells.
- Estrogenic responses were observed in APE/pyrethroid mixtures.
- Mechanism--enhanced biotransformation?

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Commercial

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  - 2005-35107-16189
- USDA-AES-UCR/RSAP
- Bay Delta Science Program


PEACE
LOVE
AND
BEER
09/25/2010

Questions??