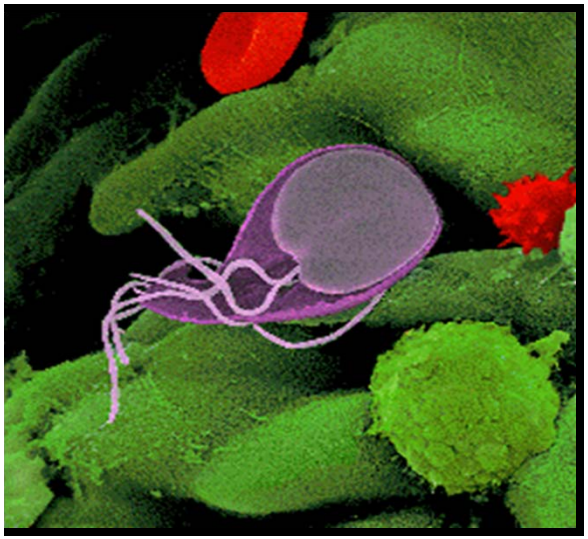
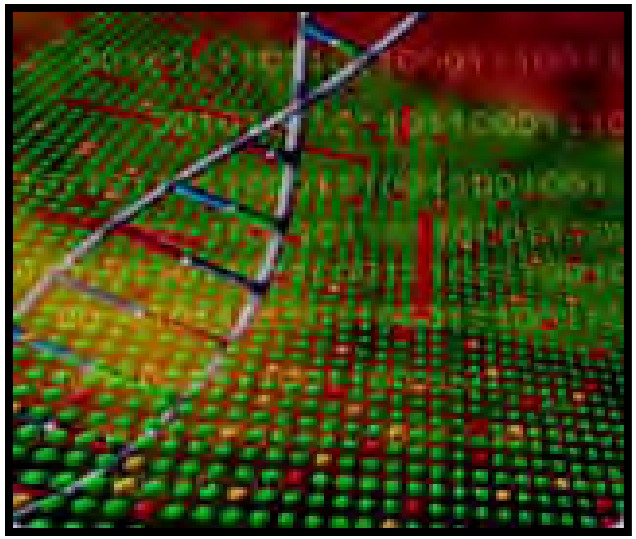
Applications of Bioassays for Recycled Water

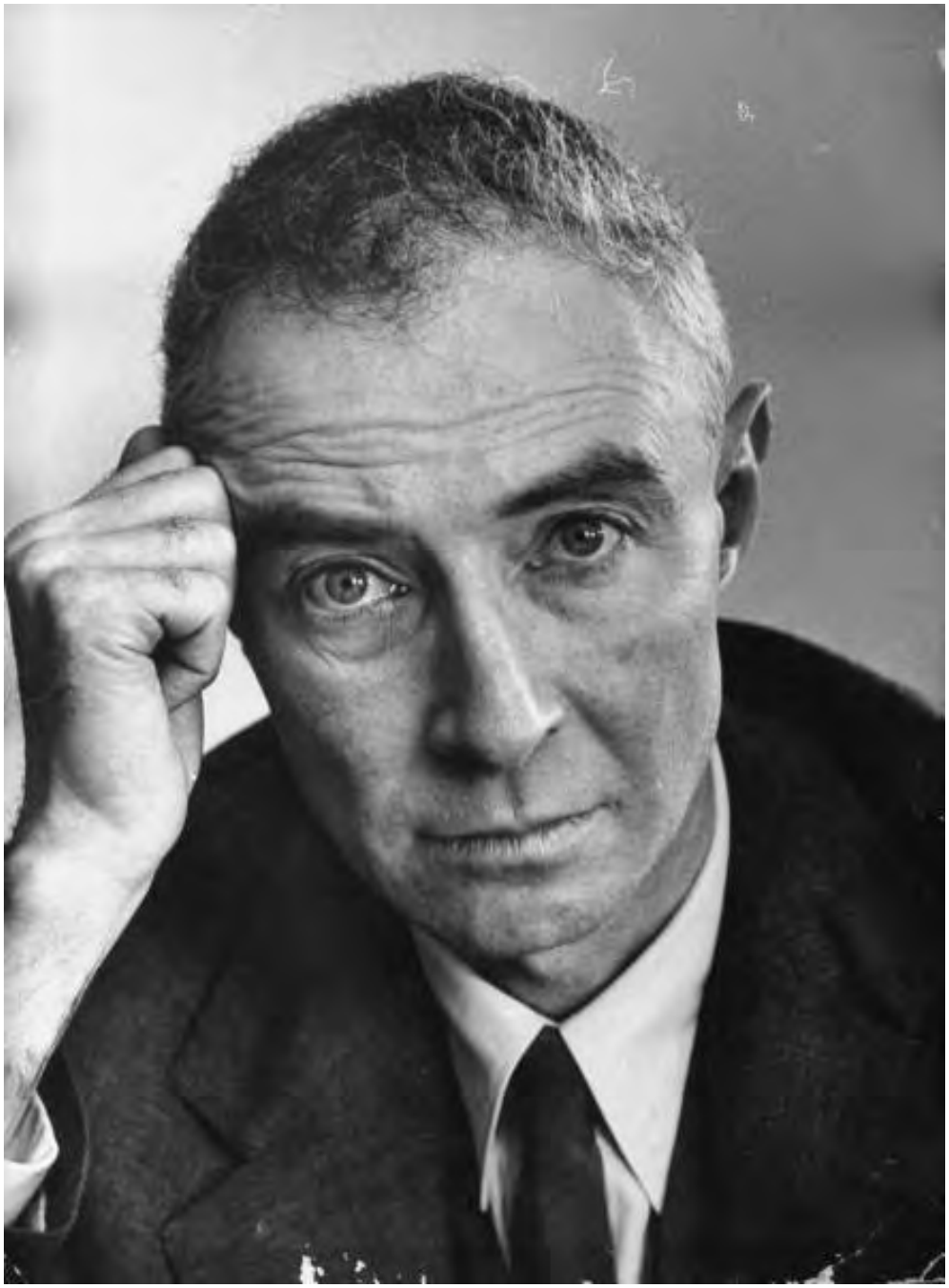

California Water Boards: Recycled Water Research Workshop

27th October 2015 – Costa Mesa

Shane Snyder, Ph.D.

Professor & Co-Director
University of Arizona

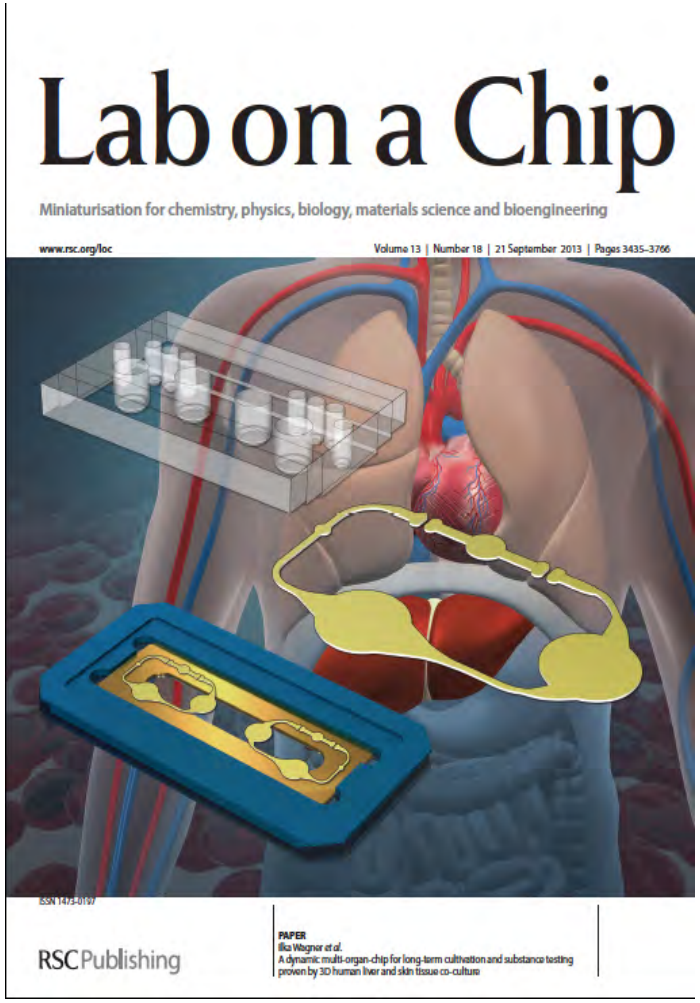
Visiting Professor
National U. of Singapore



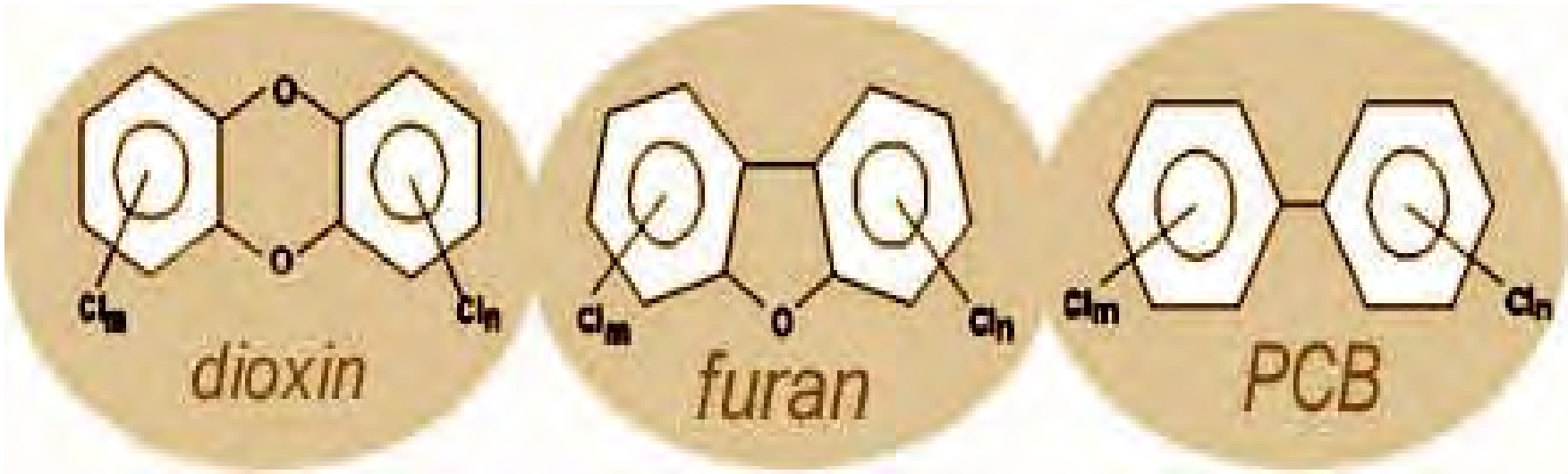
J. Robert Oppenheimer
(1904-1967)

"It is a profound and necessary truth that the deep things in science are not found because they are useful; they are found because it was possible to find them."

A Shift in Paradigm is Imminent



US EPA Already uses In Vitro Bioassays for Water Safety



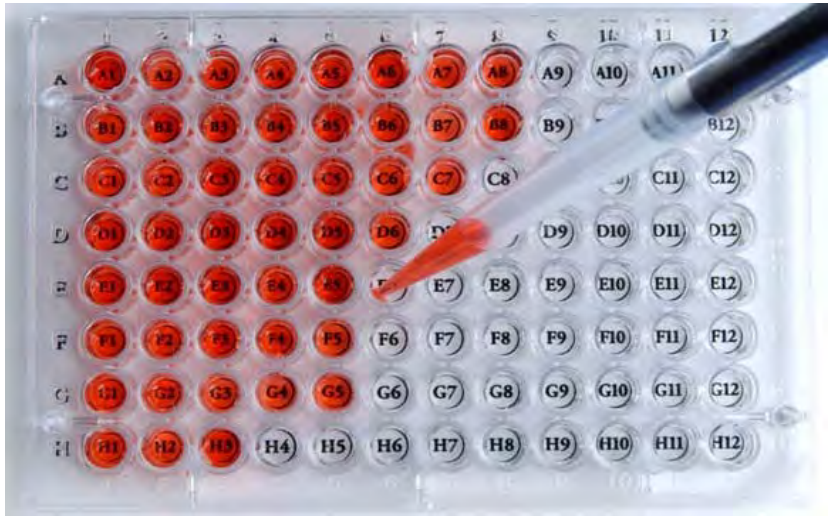
Targeted vs. Untargeted

Targeted Analytical

Known compounds
Quantitative
Individual compounds

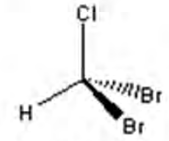
Mechanistic Bioassay

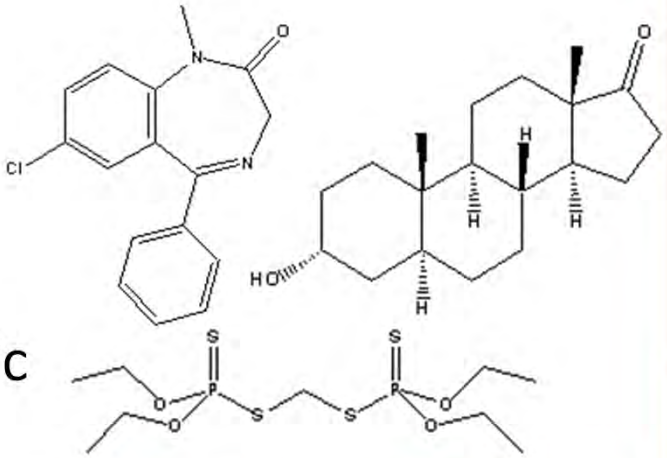
Knowns/unknowns
Semi-quantitative
Synergism/Antagonism



Why Bother With Bioassays?

- The problem …
  - Too many chemicals
  - Mixture toxicity
    - additive, synergistic, antagonistic





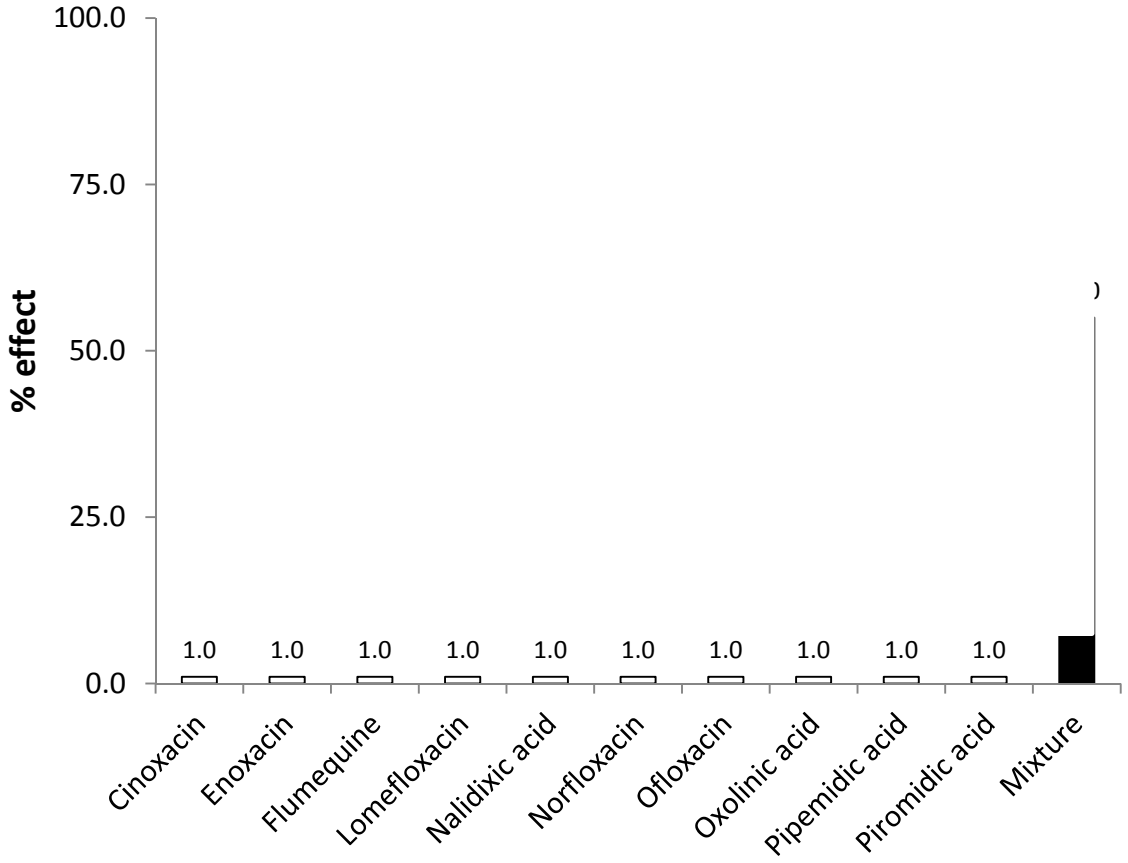
- Bioassay tools
  - Toxicity testing
  - Adverse outcome pathway
  - Bioanalytical tools
  - Water treatment monitoring
    - Especially potable reuse

Why Bother With Bioassays?

- There are too many chemicals out there to quantify them one by one analytically…
- Far too many to conduct animal testing one by one…
  - 33,000,000 animals were used in the USA alone in a single year (1988 NAS Report)
  - Animals not always good to mimic effects in humans (i.e., stomach and pharmacokinetics)
  - EPA and WHO use large uncertainty (safety) factors because of difficulties in extrapolating

Something from nothing …

10 similarly acting toxicants on bacterial luminescence



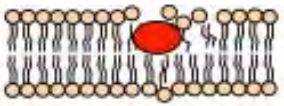
Backhaus *et al.* (2008) in Kummerer (Ed.) Pharmaceuticals in the Environment, pp. 257-276

Bioassay Endpoints

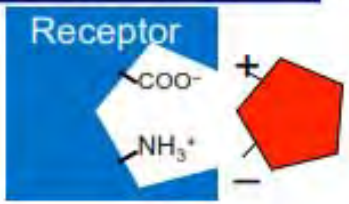
Mode-of-action based test battery

- Test systems that are indicators for relevant modes of toxic action
  - Representative examples need to be chosen from each category of modes of action

Non specific toxicity



Specific toxicity

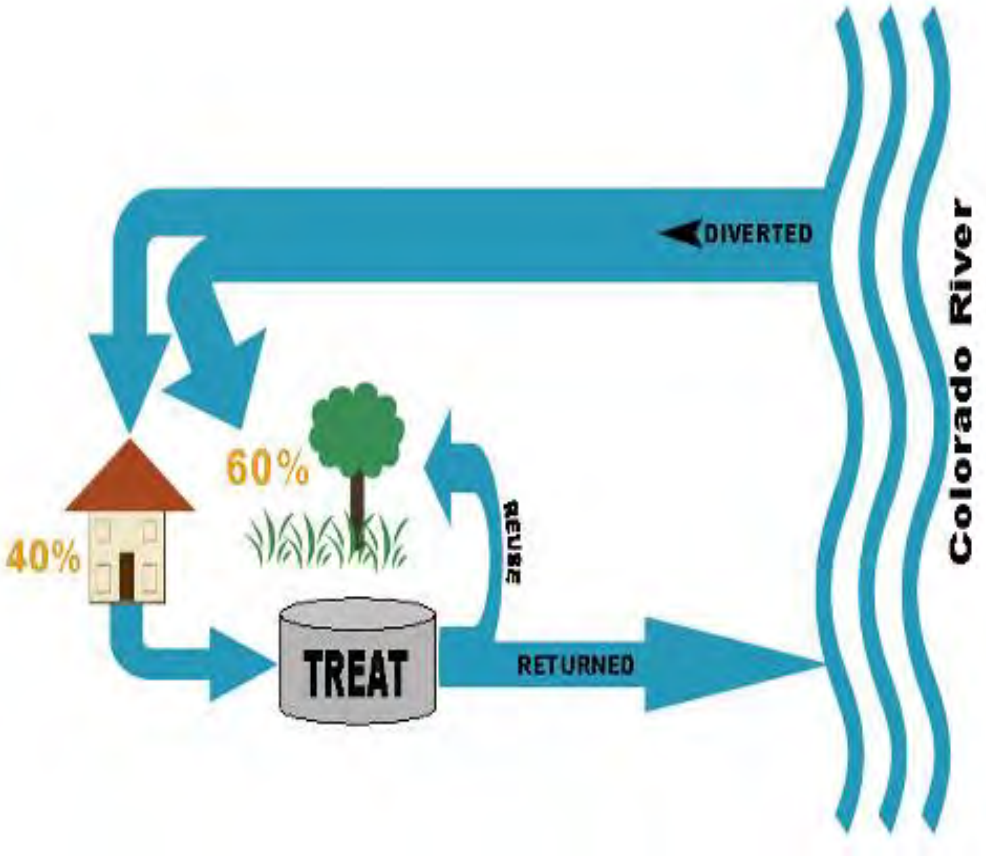


Reactive toxicity

- alternatively the response of the cell can be quantified as indirect indicator of the presence of chemicals (adaptive stress response pathways, repair and defense mechanisms)

Las Vegas – 1985

Return Flow Credit Established:
Water returned to the Colorado earns credit to withdraw an equivalent amount of drinking water



U.S. Department of the Interior—U.S. Geological Survey

SYNTHETIC ORGANIC COMPOUNDS AND CARP ENDOCRINOLOGY AND HISTOLOGY IN LAS VEGAS WASH AND LAS VEGAS AND CALLVILLE BAYS OF LAKE MEAD, NEVADA, 1992 AND 1995

Water-Resources Investigations Report 96-4266

Nevada Basin and Range Study Unit
National Water-Quality Assessment Program

By Hugh E. Bevans[1], Steven L. Goodbred[2], John F. Miesner[3], Sharon A. Watkins[1], Timothy S. Gross[4], Nancy D. Denslow[4], *and* Trenton Schoeb[4]

ABSTRACT

The Nevada Basin and Range study unit of the National Water-Quality Assessment Program, U.S. Geological Survey, in cooperation with the National Park Service, National Biological Service, and U.S. Fish and Wildlife Service, investigated the occurrence of organochlorines and semivolatile industrial compounds in the water column, bottom sediment, and carp (*Cyprinus carpio*) tissue at five sites in Las Vegas Wash and Lake Mead. Endocrine systems of carp were assessed by analyzing concentrations of female and male sex-steroid hormones, 17β-estradiol and 11-ketotestosterone, and vitellogenin (an estrogen-controlled egg protein) in blood-plasma samples. The histology of carp gonads, hepatopancreas, kidney, gill, and lower intestine were analyzed for effects that can result from endocrine disruption or exposure to toxicants.

Organochlorines (pesticides and industrial compounds) and semivolatile industrial compounds were detected in semipermeable membrane devices and bottom-sediment samples; only organochlorines were detected in carp-tissue samples. Concentrations of organochlorines were higher in Las Vegas Wash and Bay than in Callville Bay (the reference site) for the three media that were sampled. Results of a carp-tissue bioassay indicated the presence of dioxins or furans with low toxic-equivalent factors relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Las Vegas Wash and Las Vegas and Callville Bays. Patterns of necrosis observed in hepatopancreas and kidney samples from carp are consistent with long-term subchronic exposure to toxicants. Polycyclic aromatic hydrocarbons, phthalates, and phenols also were detected at higher concentrations in bottom-sediment samples from Las Vegas Bay than in a comparable sample from Callville Bay. Polycyclic aromatic hydrocarbons were detected in samples from semipermeable membrane devices from all sites.

Endocrine disruption in carp from Las Vegas Wash and Bay, as compared to Callville Bay, is evidenced by high concentrations of 11-ketotestosterone levels in blood-plasma samples of female carp in Las Vegas Wash, low concentrations in male carp from Las Vegas Bay, and low 17β-estradiol concentrations in male carp from Las Vegas Bay. The most compelling evidence of endocrine disruption is the presence of vitellogenin in blood-plasma samples of male carp from Las Vegas Wash and Bay and elevated concentrations in female carp from Las Vegas Bay.

Many of the organochlorines and semivolatile industrial compounds detected in semipermeable membrane devices, bottom sediment, and carp tissue from Las Vegas Wash and Bay have been linked to endocrine disruption in fish by previous investigations of other areas. The endocrine disruption observed in carp from Las Vegas Wash and Bay could be due to the presence of these compounds.

Aerial view of lower Las Vegas Wash and Las Vegas Bay of Lake Mead. View to the northwest, Oct. 12, 1995. Photograph by A.S. VanDenburgh.

1999 *Environ. Sci. Tech. 33(16) 2814-2829*

Analytical Methods for Detection of Selected Estrogenic Compounds in Aqueous Mixtures

SHANE A. SNYDER,*,† TIMOTHY L. KEITH,† DAVID A. VERBRUGGE,† ERIN M. SNYDER,† TIMOTHY S. GROSS,‡ KURUNTHACHALAM KANNAN,† AND JOHN P. GIESY†

National Food Safety and Toxicology Center, Department of Zoology, and Institute of Environmental Toxicology, Michigan State University, East Lansing, Michigan 48824-1311, and

can operate through a number of both direct and indirect mechanisms of action, of particular concern are those compounds that mimic endogenous estrogens. The Safe Drinking Water Act Amendments of 1995 (Bill No. S.1316) and the Food Quality Protection Act of 1996 (Bill No. P.L. 104-170), which mandate comprehensive screening for estrogenic and anti-estrogenic chemicals, are examples of the increasing public concern regarding endocrine disruption. While it is known that many natural and synthetic chemicals are estrogenic, it is unclear whether the concentrations of estrogenic agents present in the environment are sufficient to cause adverse physiological effects. One aspect of conducting human or wildlife risk assessments is an exposure assessment. This suggests the need for assays and techniques to monitor the quantity and effects of endocrine

TABLE 3. Concentrations of Selected Xenoestrogens in Water Samples (Corrected for Recovery)

location	date	NP (ng/L)	OP (ng/L)	NPE (ng/L)	E2 (pg/L)	EE2 (pg/L)
			Lake Mead			
LV Wash[a]	4/30/97	1140 ± 28	43 ± 9	8990 ± 230	2670 ± 152	480 ± 68
LV Bay	4/30/97[a]	750 ± 34	27 ± 7	4850 ± 122	2210 ± 175	520 ± 117
	9/5/97	160	nd[c]	3180	188	253
LV Marina	9/5/97	nd	nd	nd	270	nd
Saddle Island[a]	4/30/97	nd	nd	nd	nd	nd
Callville Bay	9/5/97	nd	nd	nd	nd	nd

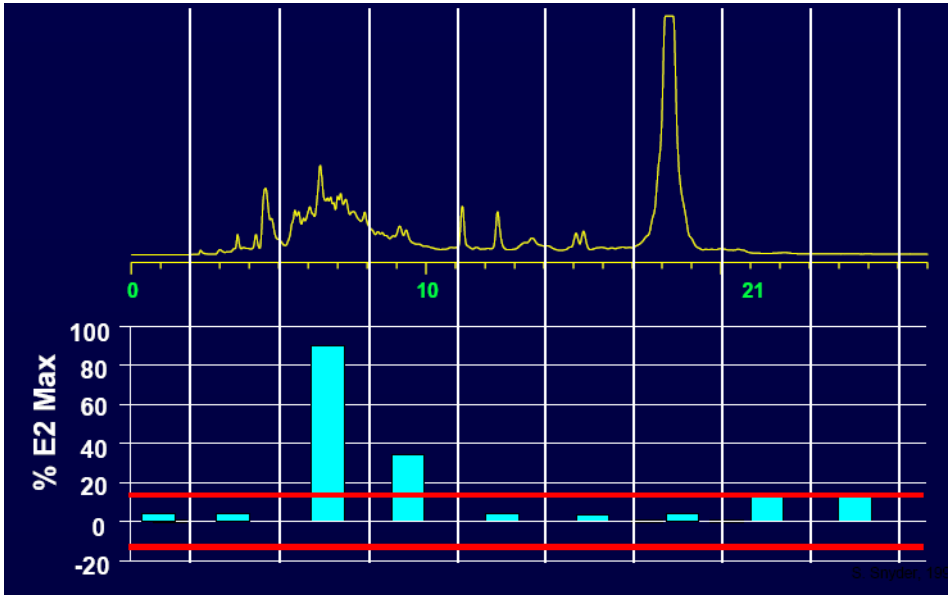
2001 Environ. Sci. Tech. 35(18) 3620-2625

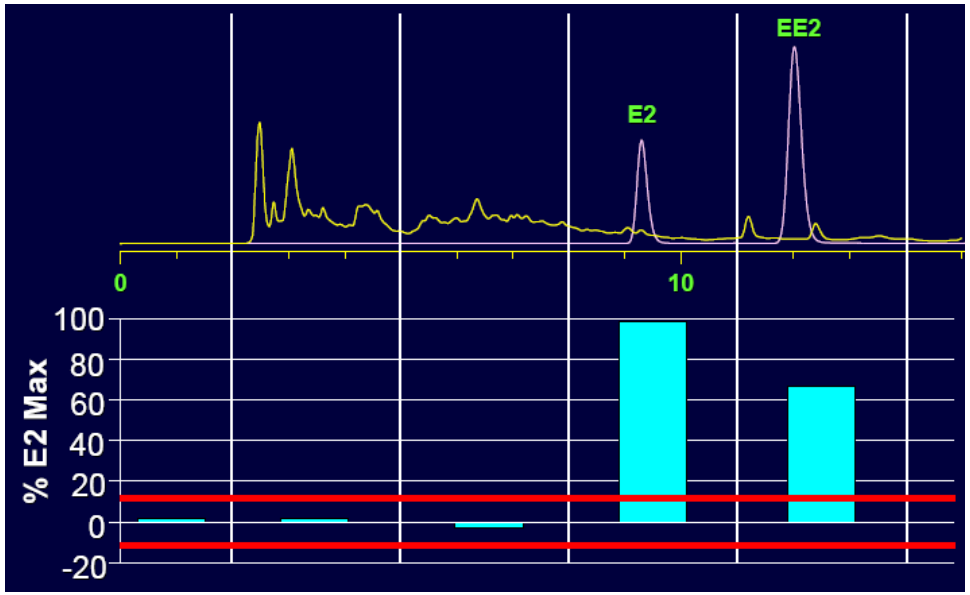
Identification and Quantification of Estrogen Receptor Agonists in Wastewater Effluents

SHANE A. SNYDER,*
DANIEL L. VILLENEUVE,
ERIN M. SNYDER, AND JOHN P. GIESY

Department of Zoology, National Food Safety and Toxicology Center, and Institute for Environmental Toxicology, Michigan State University, East Lansing, Michigan 48824-1311

2). "Endocrine-disrupting" compounds have been defined as exogenous agents that interfere with the "synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior" (*3*). It has been hypothesized that such compounds may elicit a variety of adverse effects in both humans and wildlife, including promotion of hormone-dependent cancers, reproductive tract disorders, and reduction in reproductive fitness (*1, 4*–*10*). Much of the concern has focused on compounds that are estrogen receptor (ER) agonists. These compounds have been variously referred to as "estrogenic", "estrogen-like", "environmental estrogens", or "xenoestrogens". ER agonists and antagonists have the ability to mimic or block the functions of endogenous estrogen. Effects consistent with exposure to ER agonists have been observed





ENVIRONMENTAL NEWS

Human estrogens linked to endocrine disruption

For the first time in North America, high levels of natural and synthetic hormones in municipal wastewater treatment plant effluent have been linked with endocrine disruption in fish. The study by researchers at Michigan State University's Department of Zoology indicates that human hormones, not industrial chemicals, in the effluent caused male fish to produce vitellogenin, a well-accepted indicator of endocrine disruption.

"This is a significant, if not a surprising, result," commented Gary Ankley, an EPA toxicologist who studies endocrine disrupters. The results were similar to findings published last year by U.K. researchers that identified hormones secreted in women's urine as the cause of vitellogenesis in caged fish exposed to sewage effluent in U.K. waters.

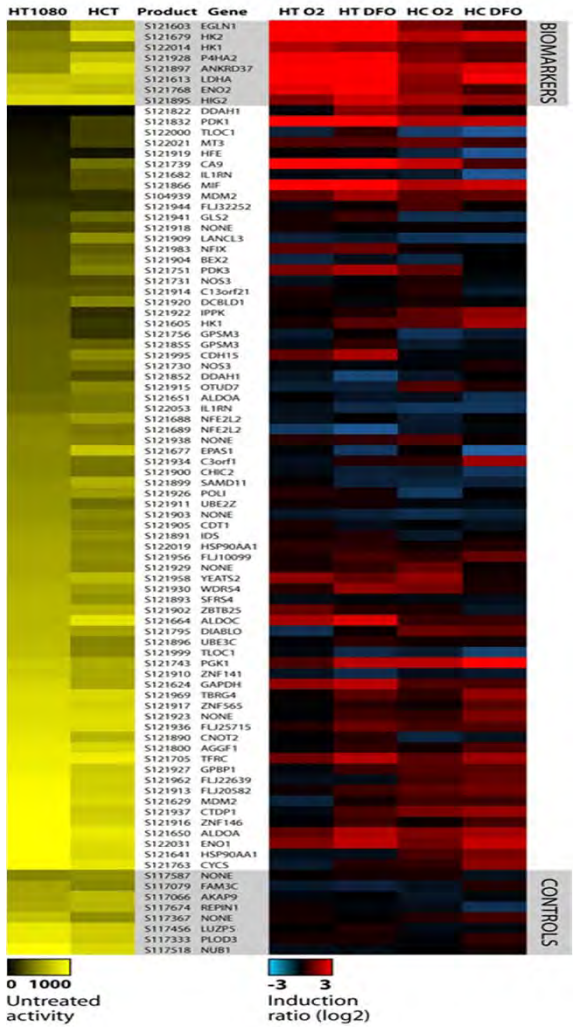
High levels of a female protein in male fish found in Lake Mead, Nev., led to a search for the cause in the effluent-dominated waters of the Las Vegas Wash. (Courtesy Shane Snyder, Michigan State University)

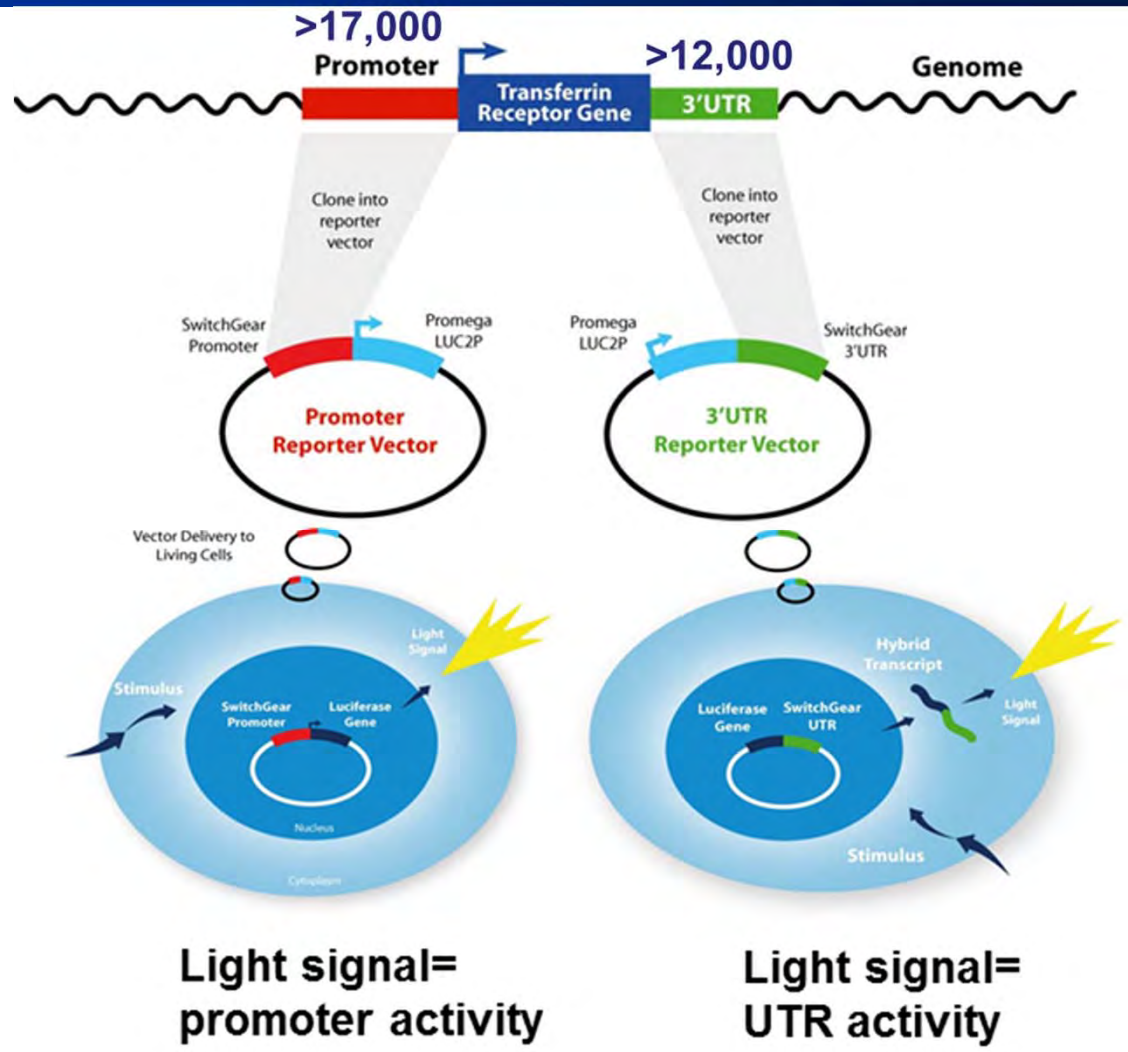
the compounds that were likely to act like estrogens in the fish. They also used an innovative method that involves solid-phase extraction and in vitro cellular bioassays to detect endocrine-modulating compounds in complex aqueous mixtures. Of the

the highest level of estrogenic activity in effluent downstream from a small plant (55,000 gal/day) with relatively few treatment processes.

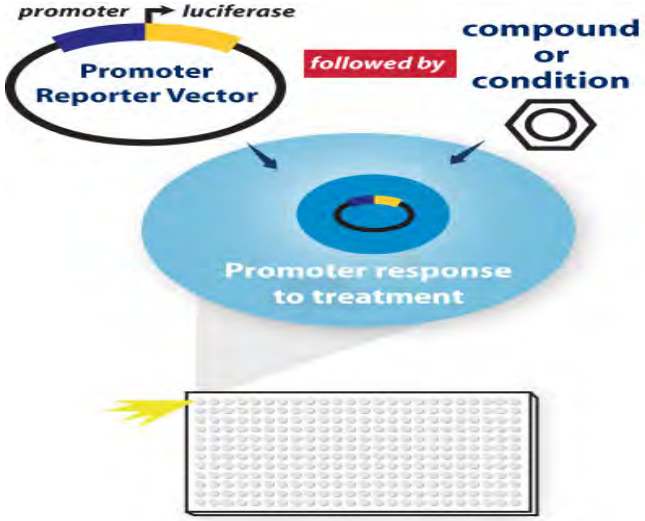
Results from a companion Michigan State study, in which caged fish were exposed to Michigan wastewater effluent, suggest

Next Generation Bioassays





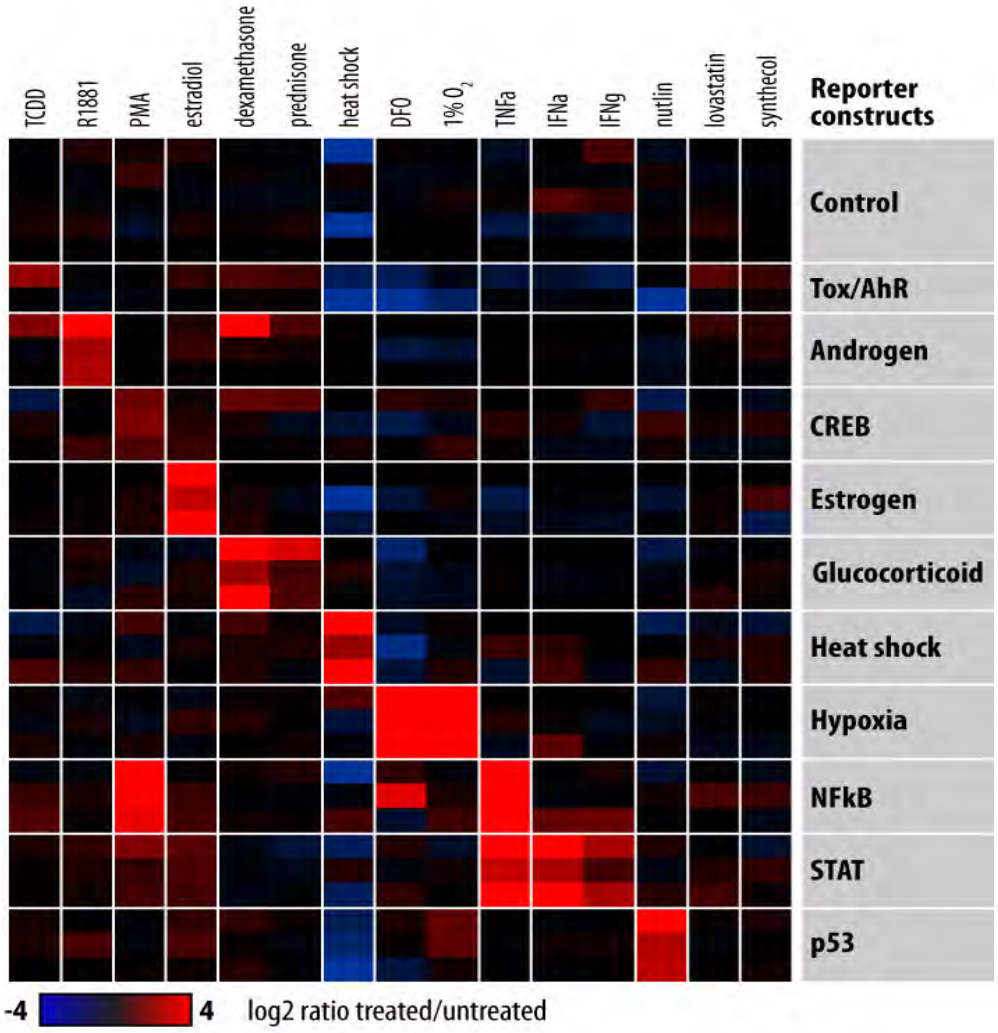
Cellular Bioassays



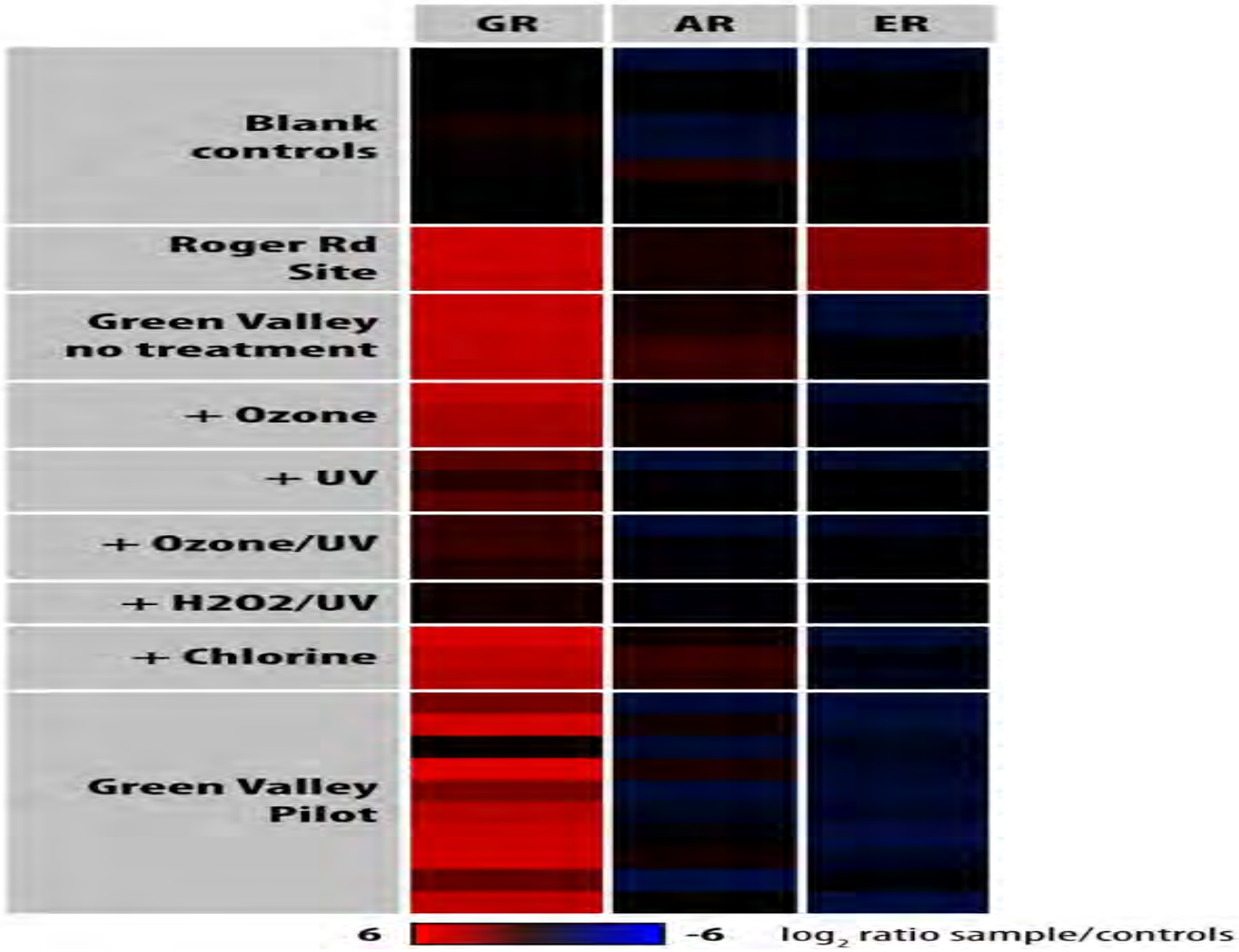
Pathway Profiling Panel
48 promoters and controls

Pathway activity readout for:

HIF1a	Hypoxia
NFkB	Inflammation
CREB	cyclic-AMP
HSF1	Heat shock
p53	DNA damage, apoptosis
STAT	Interferon
SREBP	Cholesterol biosynthesis
ER	Estrogen
AR	Androgen
GR	Glucocorticoid
AhR	Toxicity



Glucocorticoids (GR)

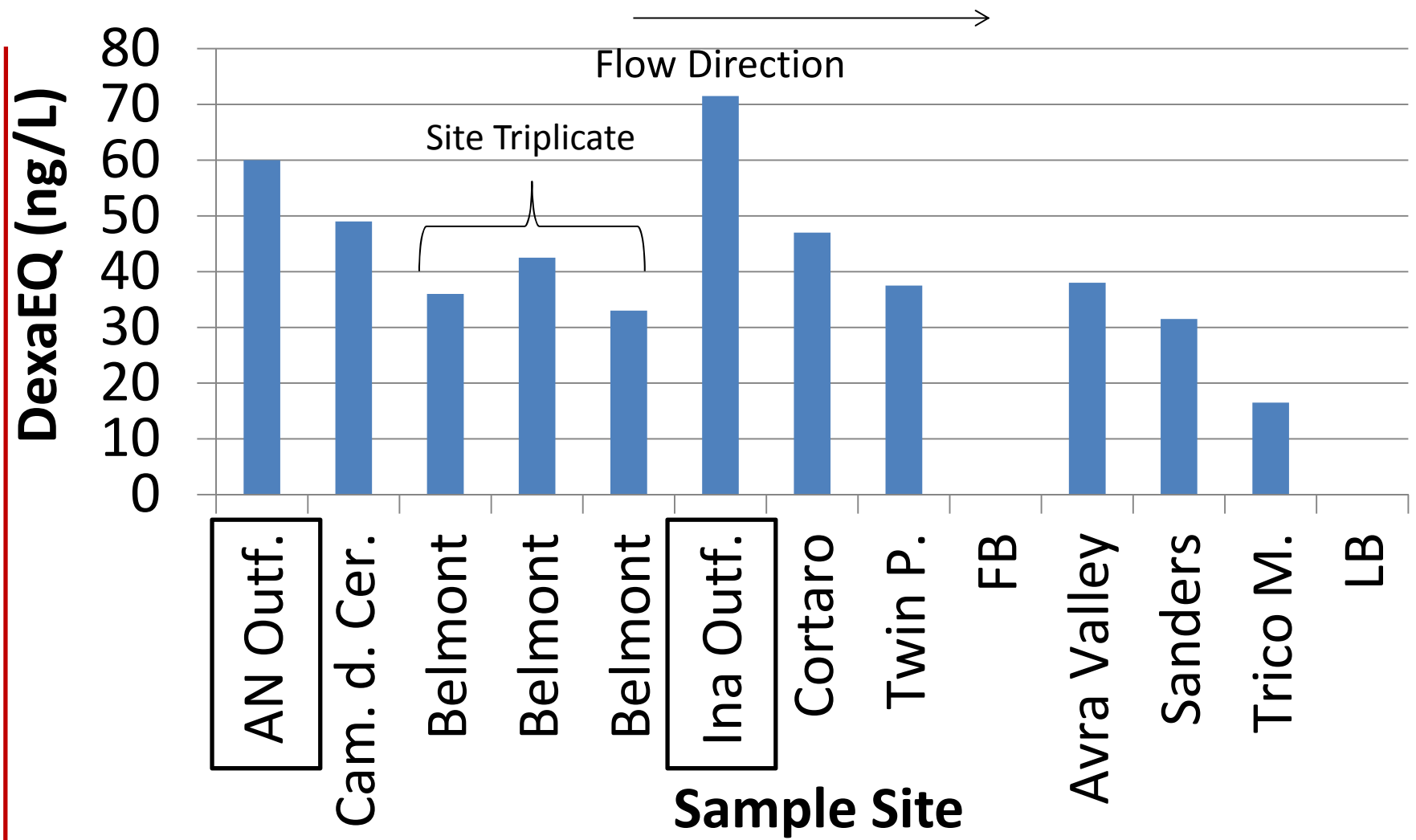


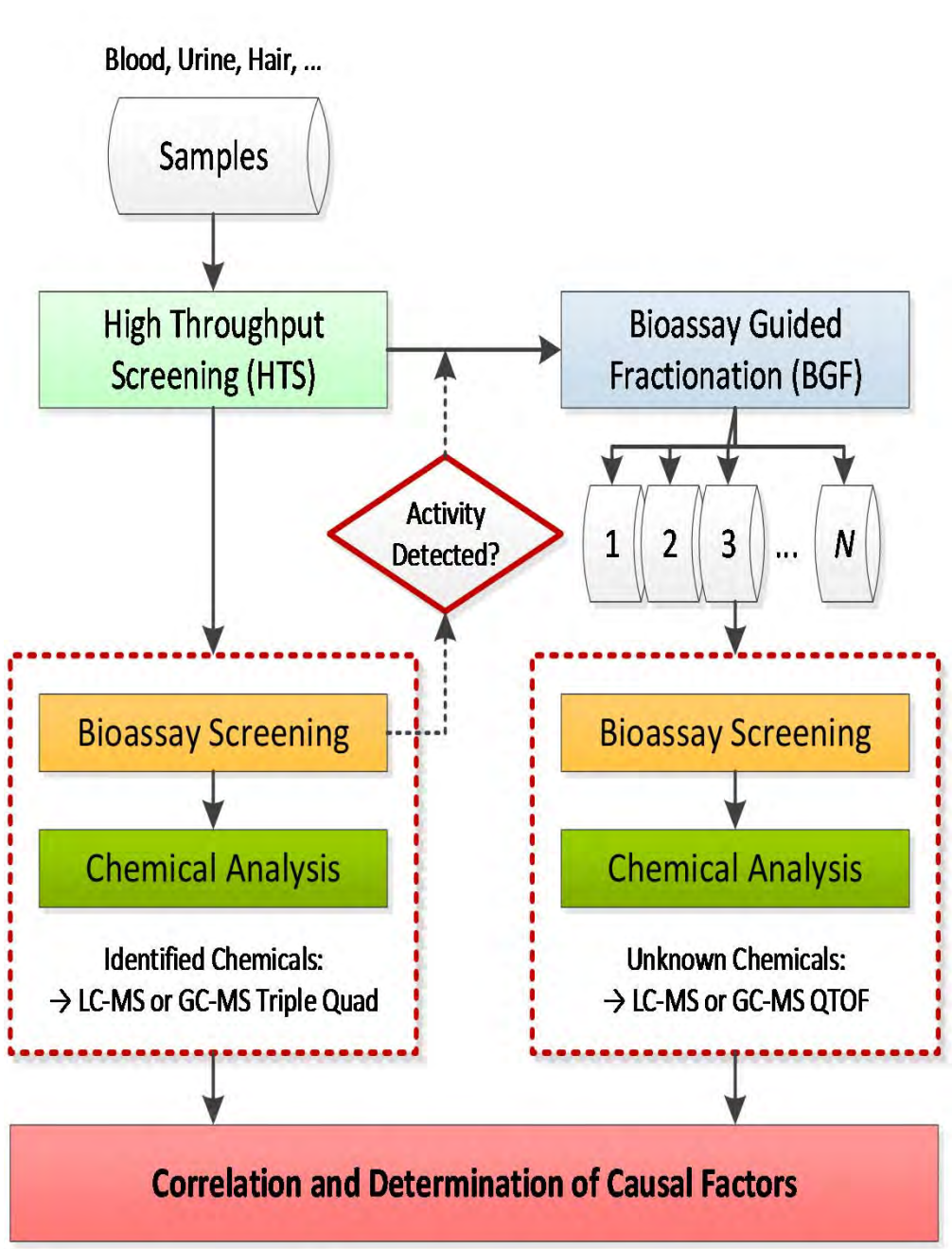
Glucocorticoids (GR)

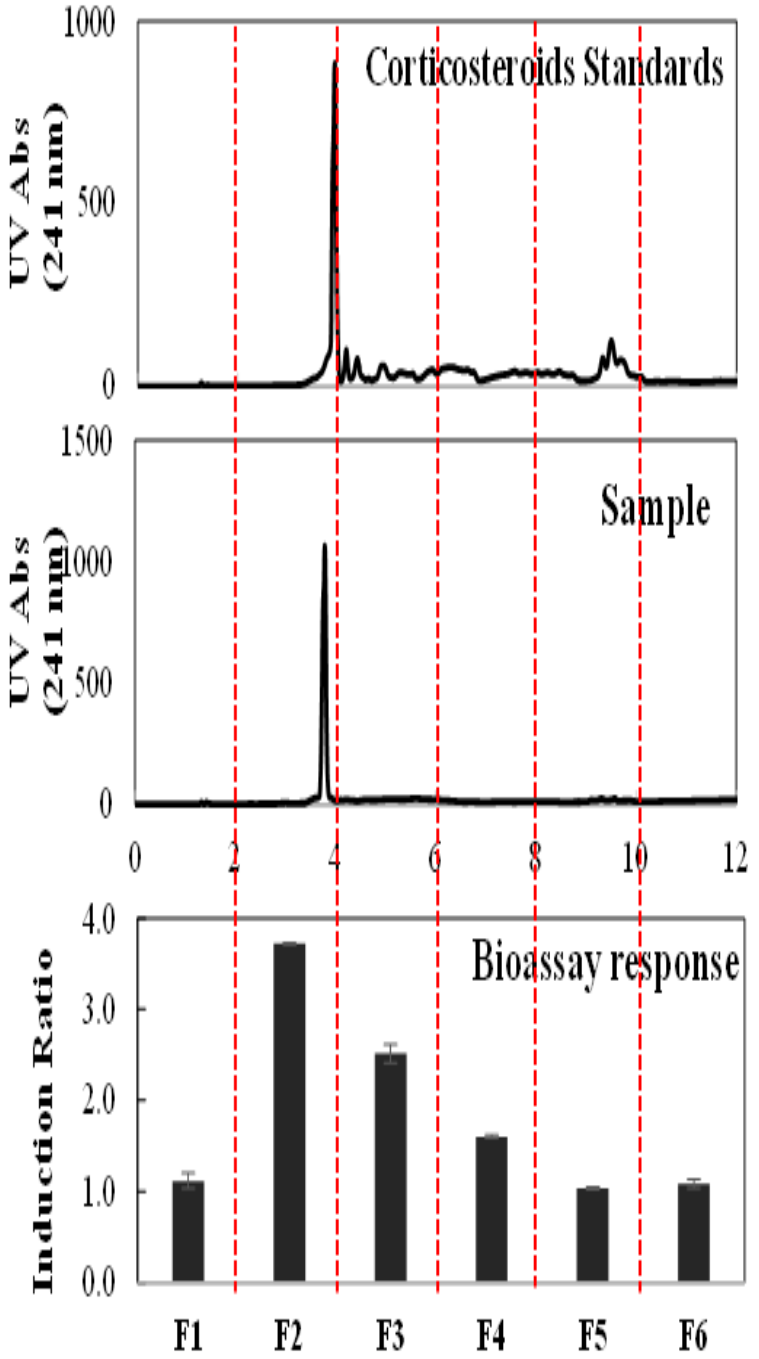
- Natural & Synthetic
- Used for human diseases such as severe allergies, skin problems, asthma, and arthritis
- Used as veterinary medicine to restore muscle strength and as growth promoters to increase muscle size

Santa Cruz River Sampling Sites

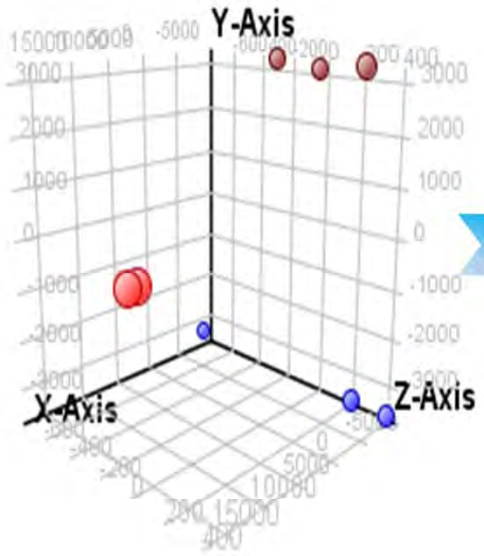
GR Cellular Activity

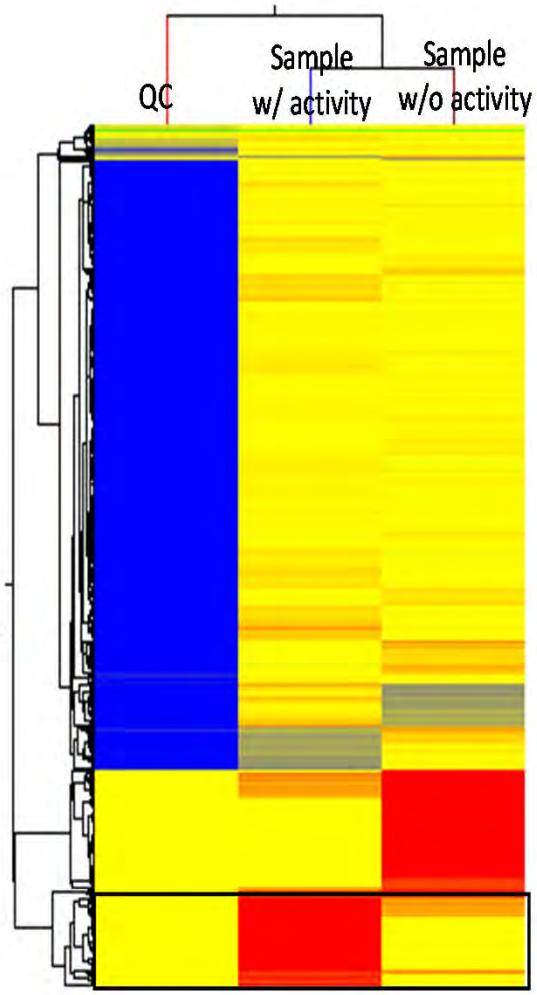



Blood, Urine, Hair, ...
Samples
High Throughput Screening (HTS)
Bioassay Guided Fractionation (BGF)
Activity Detected?
1
2
3
...
N
Bioassay Screening
Chemical Analysis
Identified Chemicals:
→ LC-MS or GC-MS Triple Quad
Bioassay Screening
Chemical Analysis
Unknown Chemicals:
→ LC-MS or GC-MS QTOF
Correlation and Determination of Causal Factors


Corticosteroids Standards
UV Abs (241 nm)
1000
500
0
Sample
1500
1000
500
0
0
2
4
6
8
10
12
Bioassay response
Induction Ratio
4.0
3.0
2.0
1.0
0.0
F1
F2
F3
F4
F5
F6

Principal Component Analysis

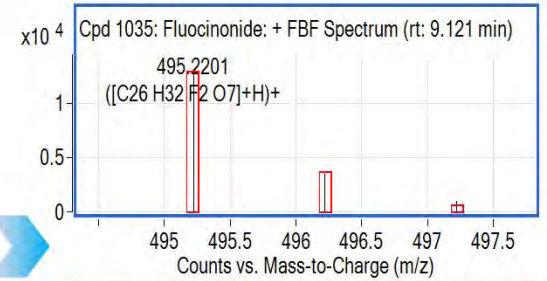


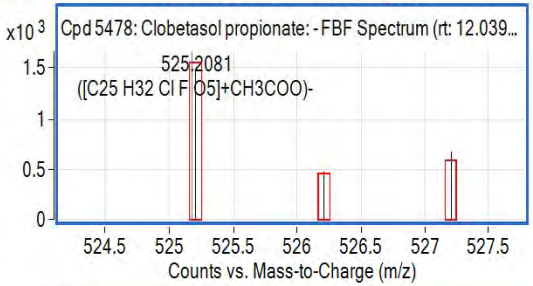


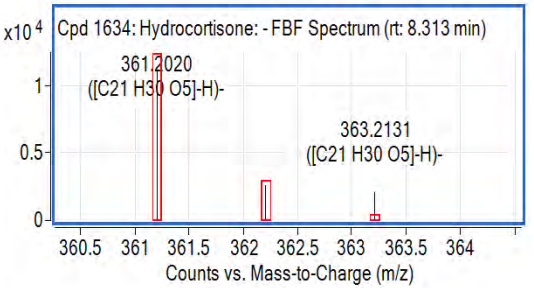
Color by normalized abundance

- QC
- Sample w/ activity
- Sample w/o activity

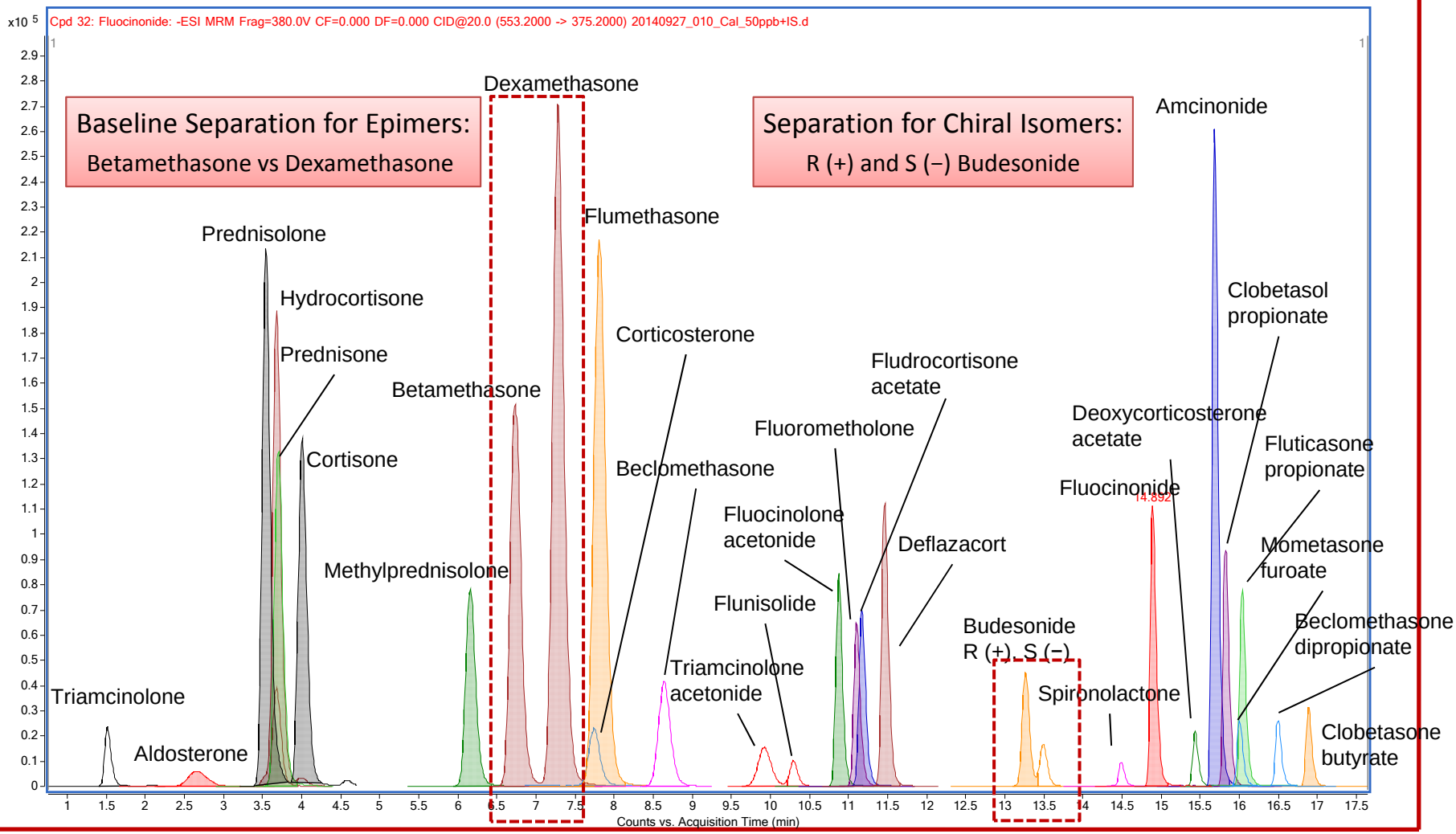
Datadase Search & Compounds Identification



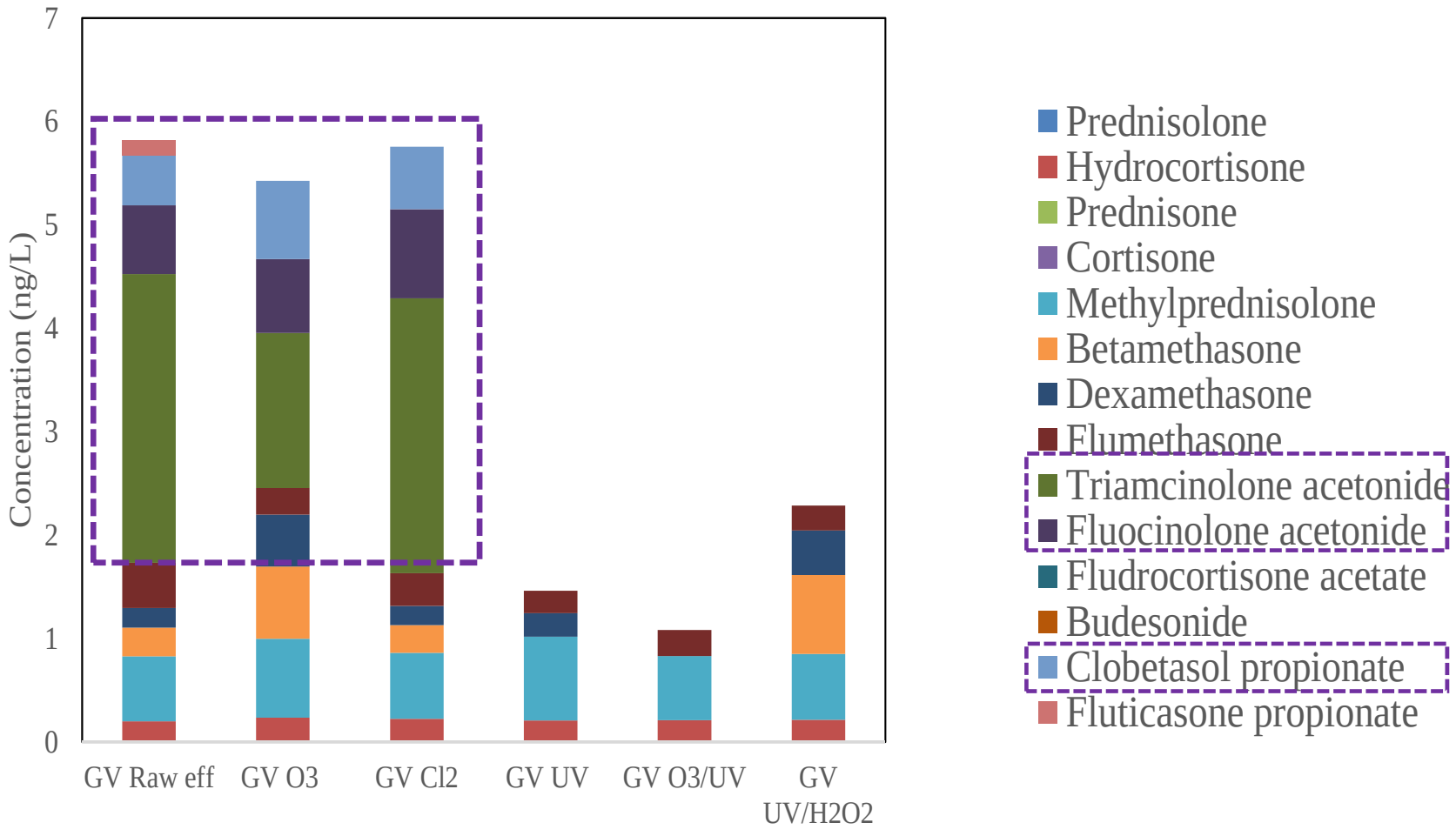




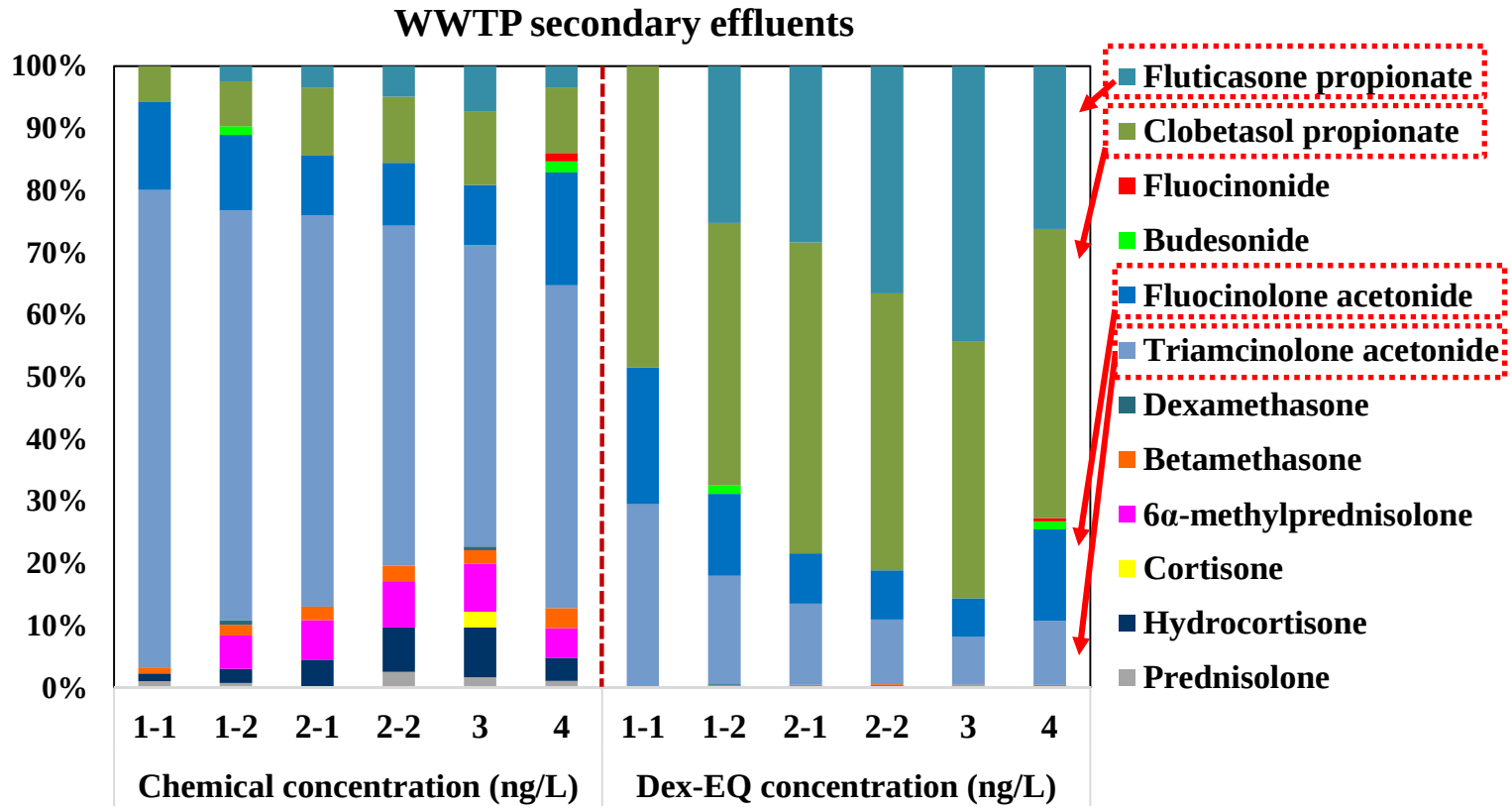
GR Mass Spectrometric Analysis



Distribution of GRs



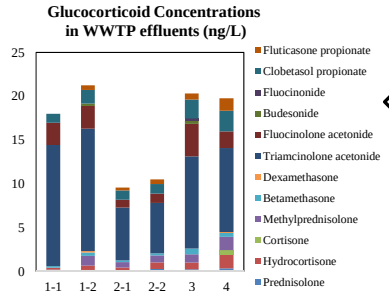
Prioritize GCs monitoring effort

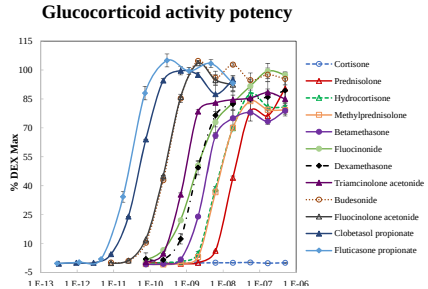


Bioassay based prioritization monitoring effort help selecting the most important and health related chemicals

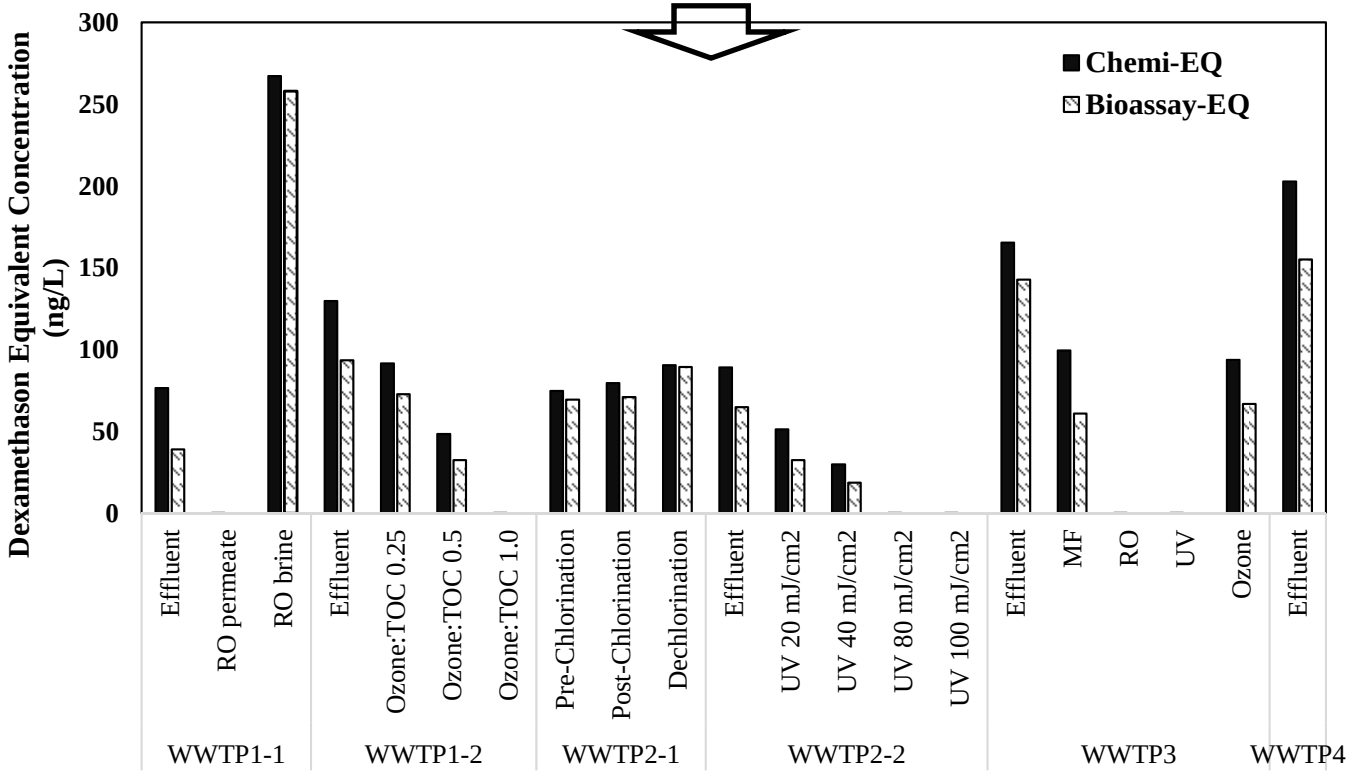
Mass-Activity Balance

Concentrations





Activities



Chemical analysis match with toxicological effect

UPDATE
National Toxicology Program
U.S. Department of Health and Human Services

July 2014

Headquartered at the
National Institute of Environmental
Health Sciences • NIH-HHS

High-throughput toxicity screening produces human-relevant results

By Thomas Burns Jr., reprinted from Environmental Factor, June 2014

Using *in vitro* and *in silico* testing in primary human cell systems, scientists reported in the May issue of the journal Nature Biotechnology bioactivity profiles for 776 unique and diverse chemicals with potential for human exposure. The use of a human-relevant system may provide a rapid and accurate screening method to prioritize chemicals for further toxicity testing or to identify new pharmaceutical activities.

"This is the first data manuscript in the field of high throughput toxicity screening [HTS] on such a large number of chemicals to be published in one of the Nature journals," said Nicole Kleinstreuer, Ph.D., the paper's lead author, who is now a contractor supporting the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). "It demonstrates the utility of HTS assays that use human primary cells to elucidate mechanisms of action and predict toxicities for a diverse set of chemicals."

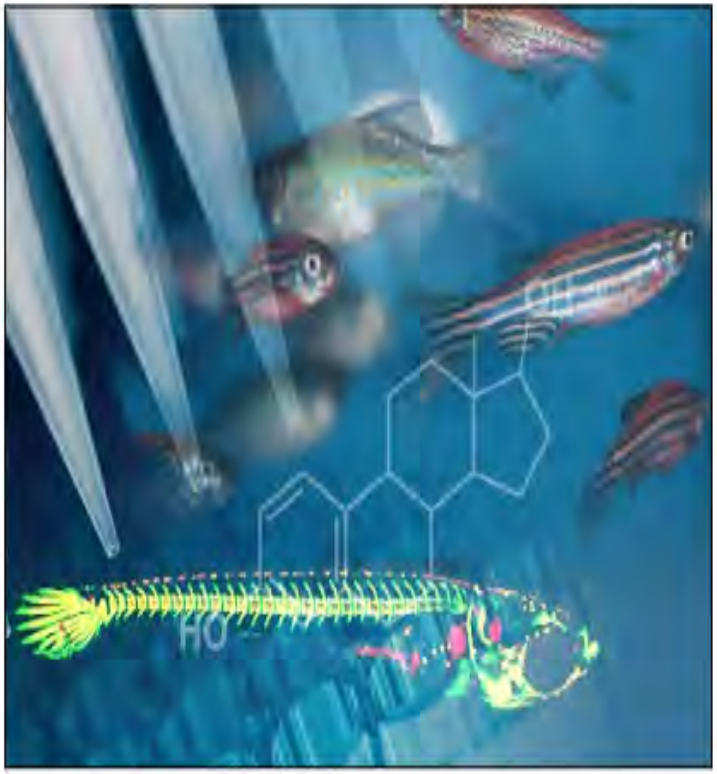
UPDATE
National Toxicology Program
U.S. Department of Health and Human Services

July 2014

Headquartered at the
National Institute of Environmental
Health Sciences • NIH-HHS

Small fish may offer big opportunities for future toxicity testing

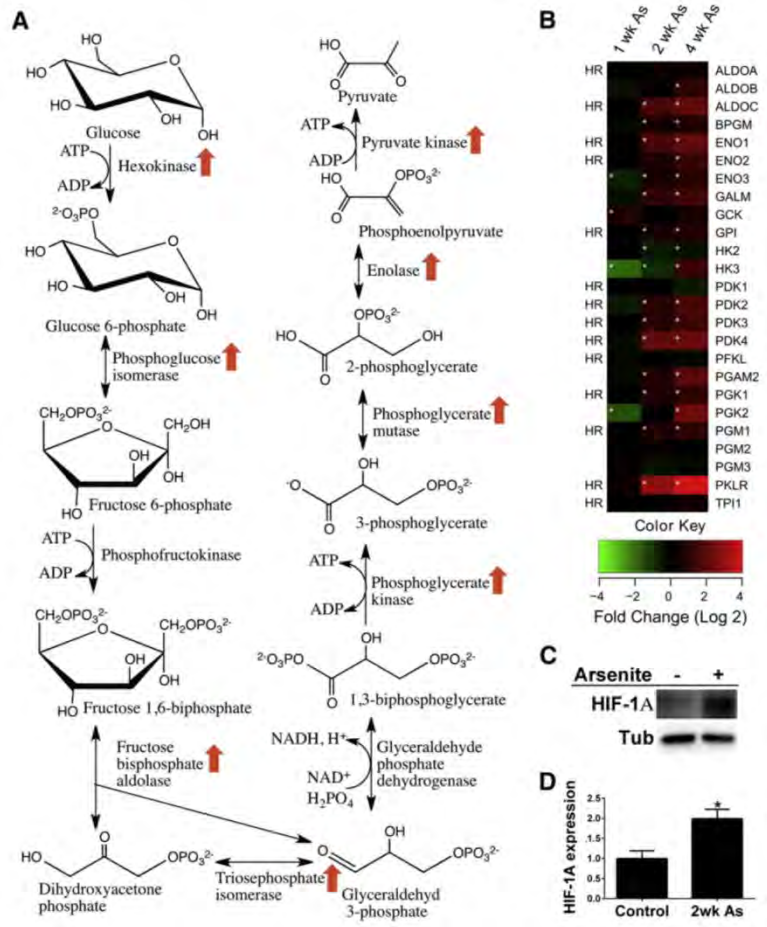
By Catherine Sprankle, reprinted from Environmental Factor, June 2014

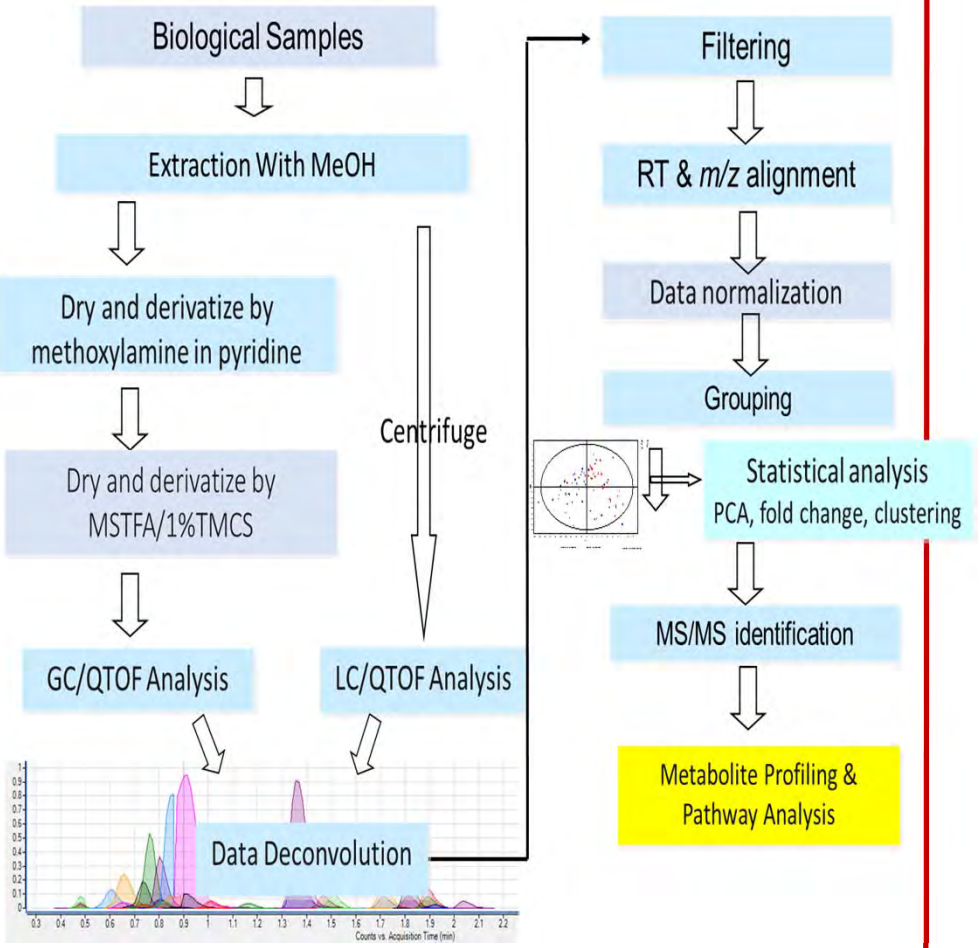


The conference concluded May 6 with a discussion session. Workshop participants noted that the suitability of small fish species for toxicity testing, particularly their practical advantages, needs to be brought to the attention of other audiences, including industry, regulators, scientists in other disciplines, and the general public.

Topics identified for further exploration included the effective application of fish study data for better understanding of chemical safety, and integration of fish data with complementary information from other types of toxicity studies. Other needs included clarification of the relationship between chemical treatment, uptake and metabolism, and the observed effects in fish models.

Metabolomic Approach





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Our Approach – Beas 2B w/wo H1F1A knockout

Where does this leave us?

1. Attempts to reduce all substances to less than detectable are FUTILE and energy intensive.
2. There is ZERO possibility that the current paradigm used for risk assessment/regulatory determination will be able to address those substances already identified, let alone the unknowns.
3. Chemicals will ALWAYS exist as complex mixtures, yet toxicity tests are conducted with single chemicals and high-doses.
4. Animal models often do not model human health.
5. Rapid bioassays is the ONLY answer for comprehensive evaluation of water toxicity.

Critical Research Needs

1. Most procedures rely on SPE/concentration
   a) Highly polar, volatile, and hydrophobic difficult
   b) pH of sample impacts ionization/extraction
   c) 1,4-dioxane, perchlorate, and NDMA examples
2. More QA/QC required for bioassays
   a) Lack of surrogates for bioassays
   b) Nontargeted analytical limited by ion/volatility/conditions in general
   c) Conducting automated SPE with microfluidic transfers should be explored

Critical Research Needs

1. More validation of S&I approach at full-scale
2. More consideration of sampling bias
   a) Diurnal, location, holding times, etc.
   b) Especially in regard to bioassay methods
3. Rational discussion regarding appropriate detection limits to better optimize methods
4. Screening of RO Brines for initial characterization of contaminant occurrence
   a) ≈5-10x concentration of source water
   b) Passive samplers for bioaccumulation studies and composite samples for hydrophilic contaminants

SNYDER RESEARCH GROUP

PIONEERING RESEARCH REGARDING DETECTION, TREATMENT, AND HEALTH RELEVANCE OF ENVIRONMENTAL CONTAMINANTS

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