Bioanalytical Screening for Constitutents of Emerging Concern (CECs)

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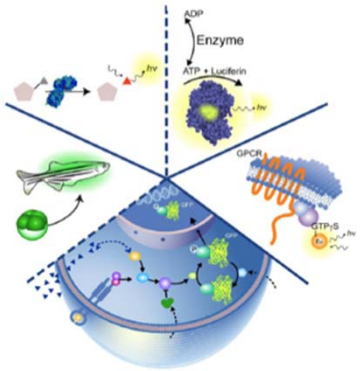
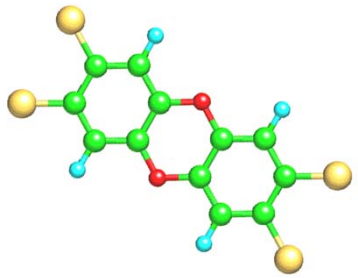
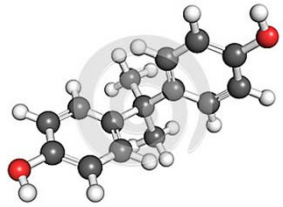
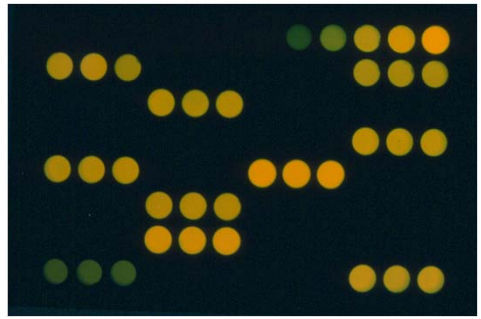
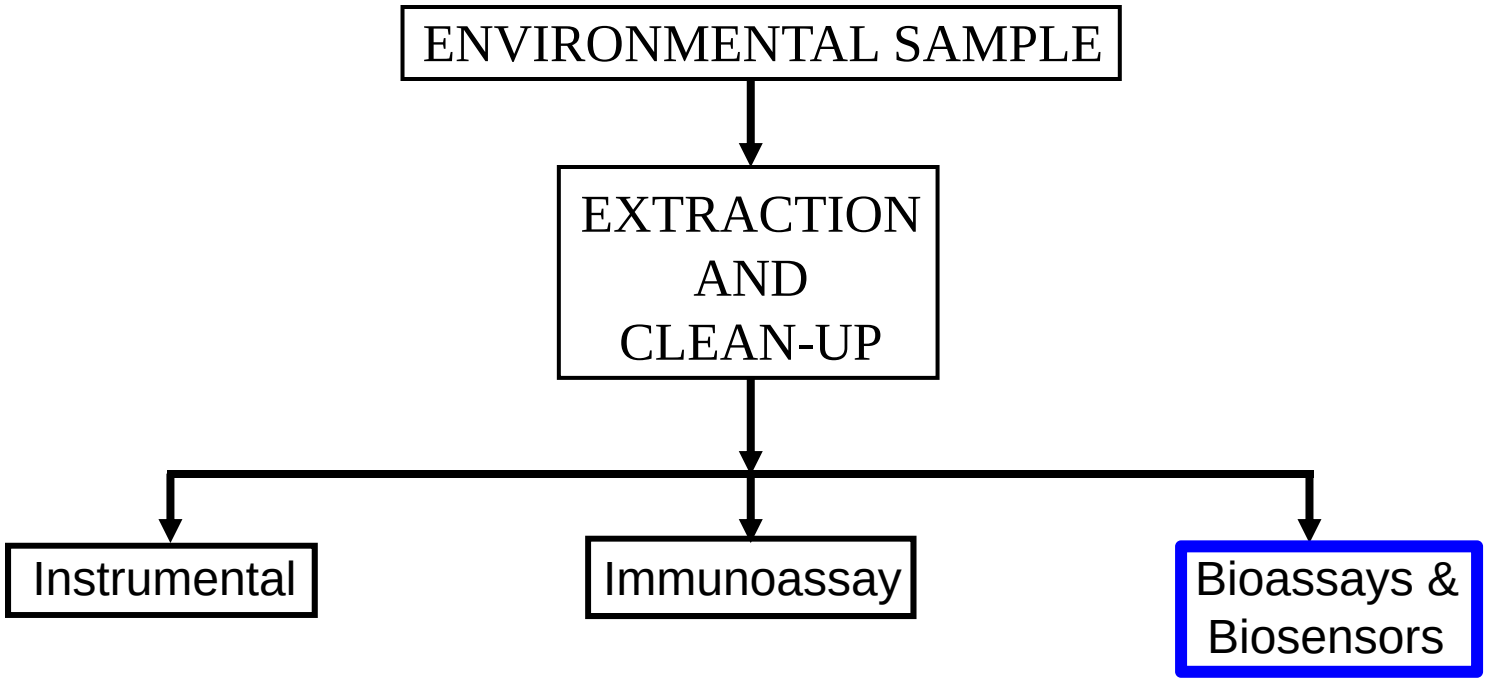








Screening and Monitoring Approaches for Environmental Chemicals of Concern (Known and Unknown)



Bioassays can't be comprehensive – some mechanisms and assays are not amenable to HTS, multifactorial mechanisms are problematic.

What are appropriate targets for useful bioassays?

Environmental Monitoring With In Vitro Bioassays

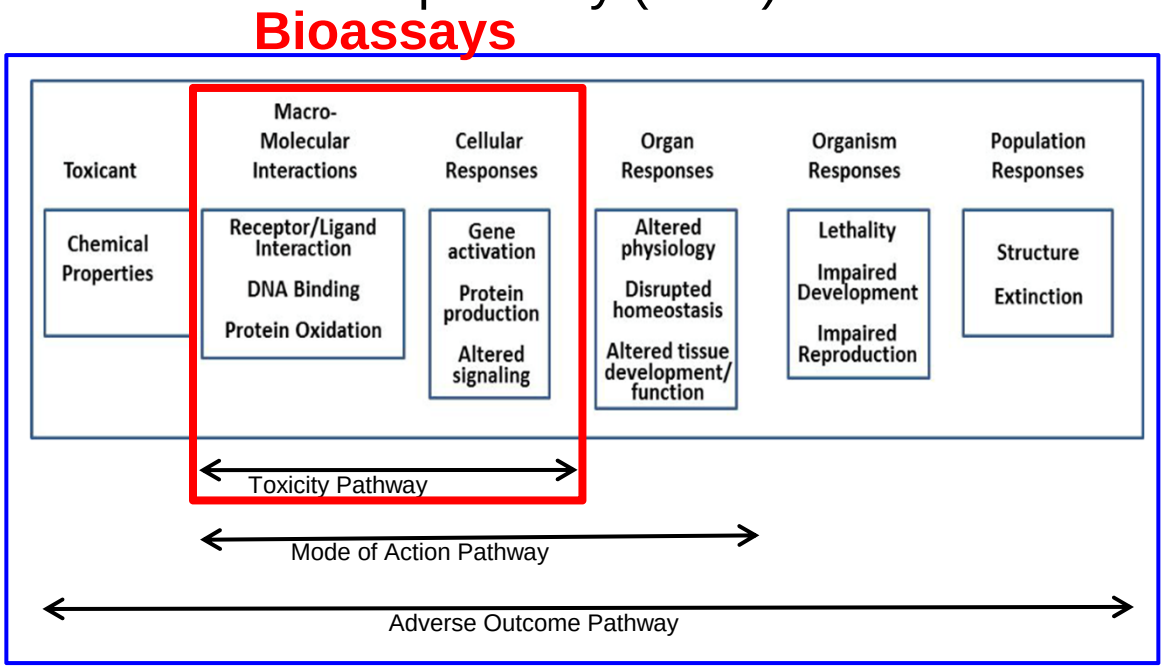
Chemical/Chemical Class Detection Bioassays (selective screening)

Few chemical selective bioassays available (dioxin-like chemicals)

"Hazardous" Chemical Detection Bioassays (open-ended Screening)

Requires some qualitative/quantitative relationship with risk

- First step requires relating the bioassay and bioassay result to an adverse outcome pathway (AOP).



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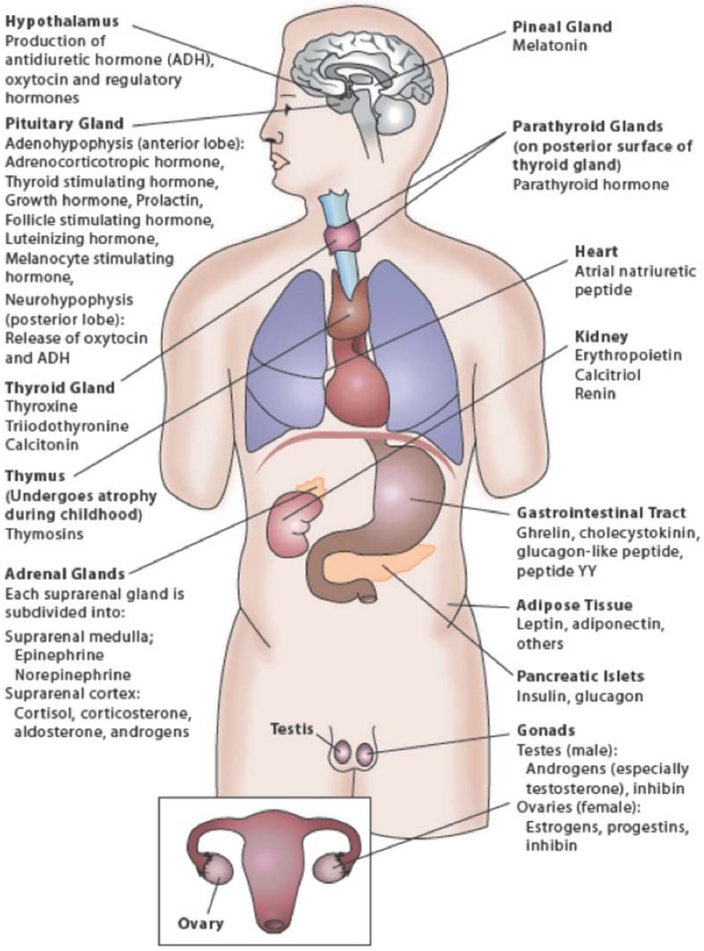
Requires some qualitative/quantitative relationship with risk

- First step requires relating the bioassay and bioassay result to an adverse outcome pathway (AOP).
- Second, confirm relationship the concentration-response of the bioassay with the dose-response for adverse health outcomes produced *in vivo* (animals or humans). Compare blood levels versus bioassay exposure concentrations.
- A given bioassay responds to chemicals that act through a common mechanism and/or AOP.

Example of a bioassay for environmental monitoring?

Environmental Screening Bioassay Example: Endocrine (Hormone) Disrupting Chemicals (EDCs)

Endocrine Glands/Organs - Hormones

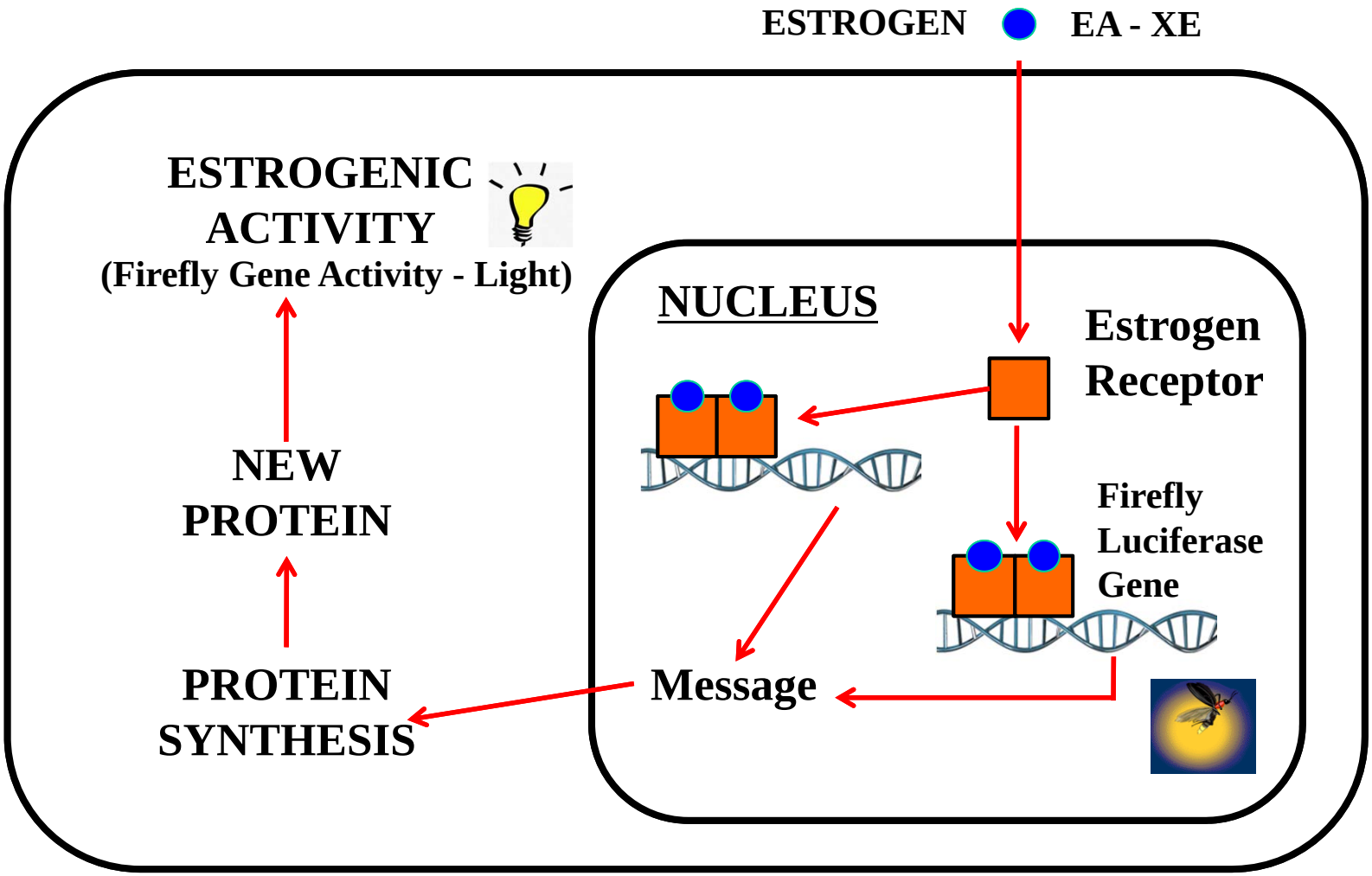


Definition of EDCs (IPCS, 2002)

*"An **endocrine disruptor** is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations."*

*"A **potential endocrine disruptor** is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations."*

Mechanism of Estrogen Action:
Bioassay for Estrogenic Chemicals (Xenoestrogens (XE))



Estrogen-Responsive Cell

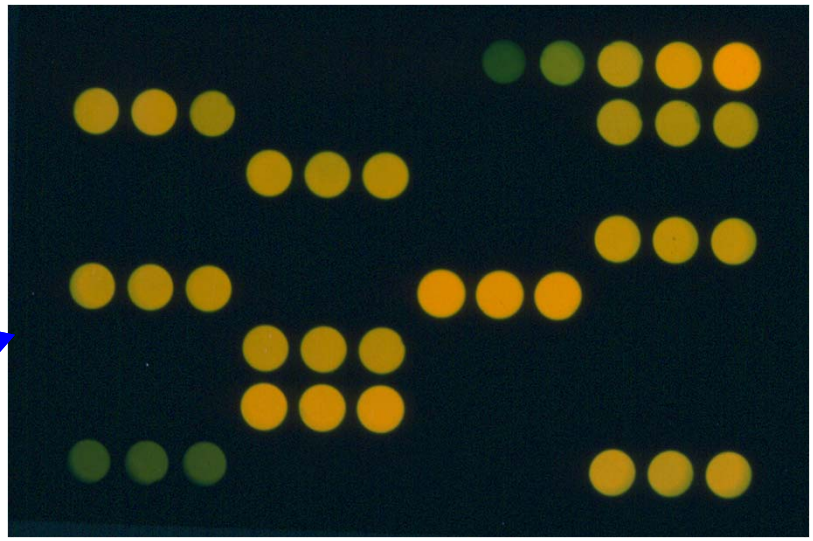
EA - Estrogen Active (chemical)

Hormone (Estrogen) Receptor Cell Bioassay Procedure

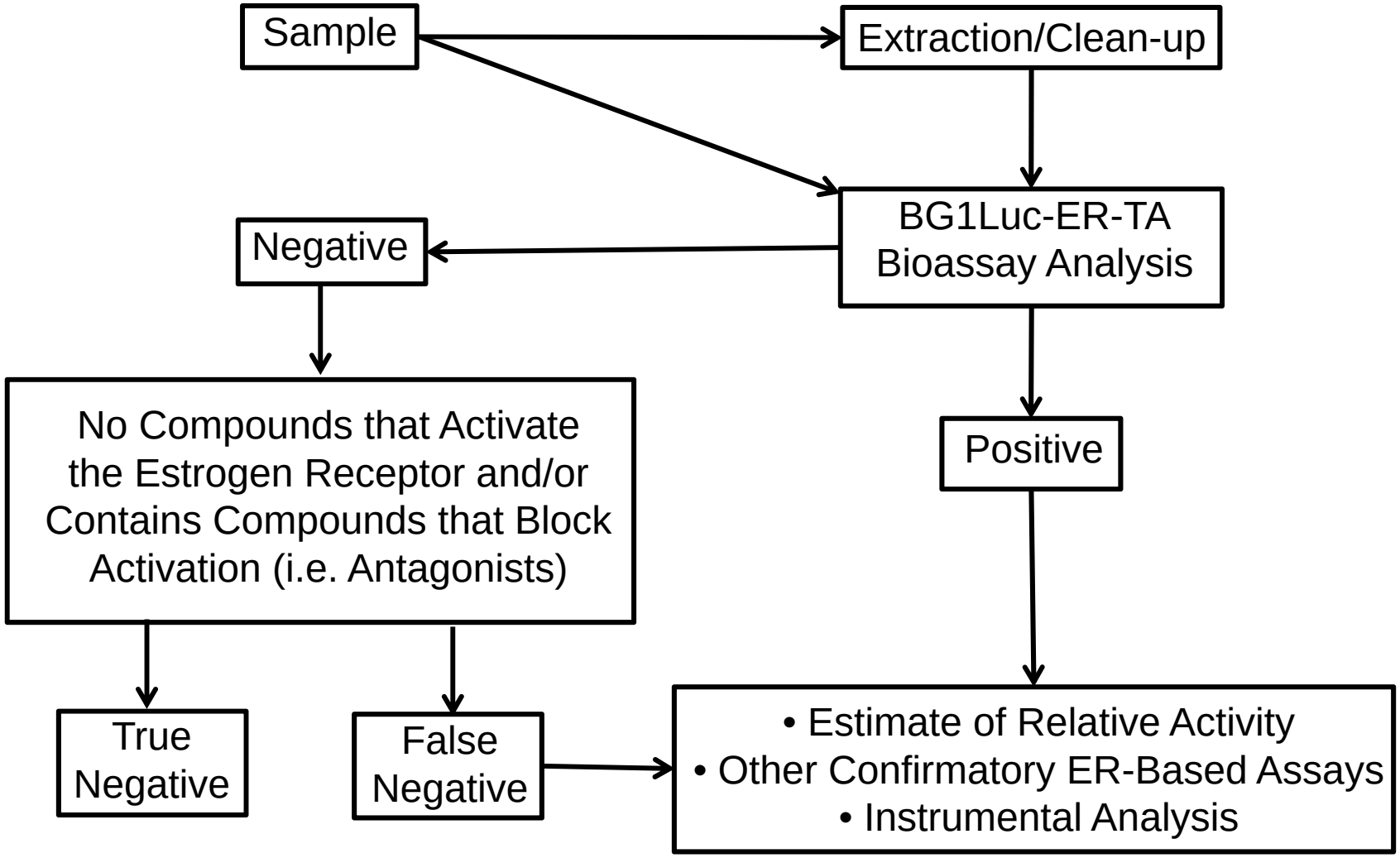
Recombinant human liver cells are grown in 96-well microplates for 3-4 days

Chemicals or extracts are added to each well and cells incubated for 24 hours

Amount of light produced is directly proportional to the concentration of estrogenic chemical added to the cells

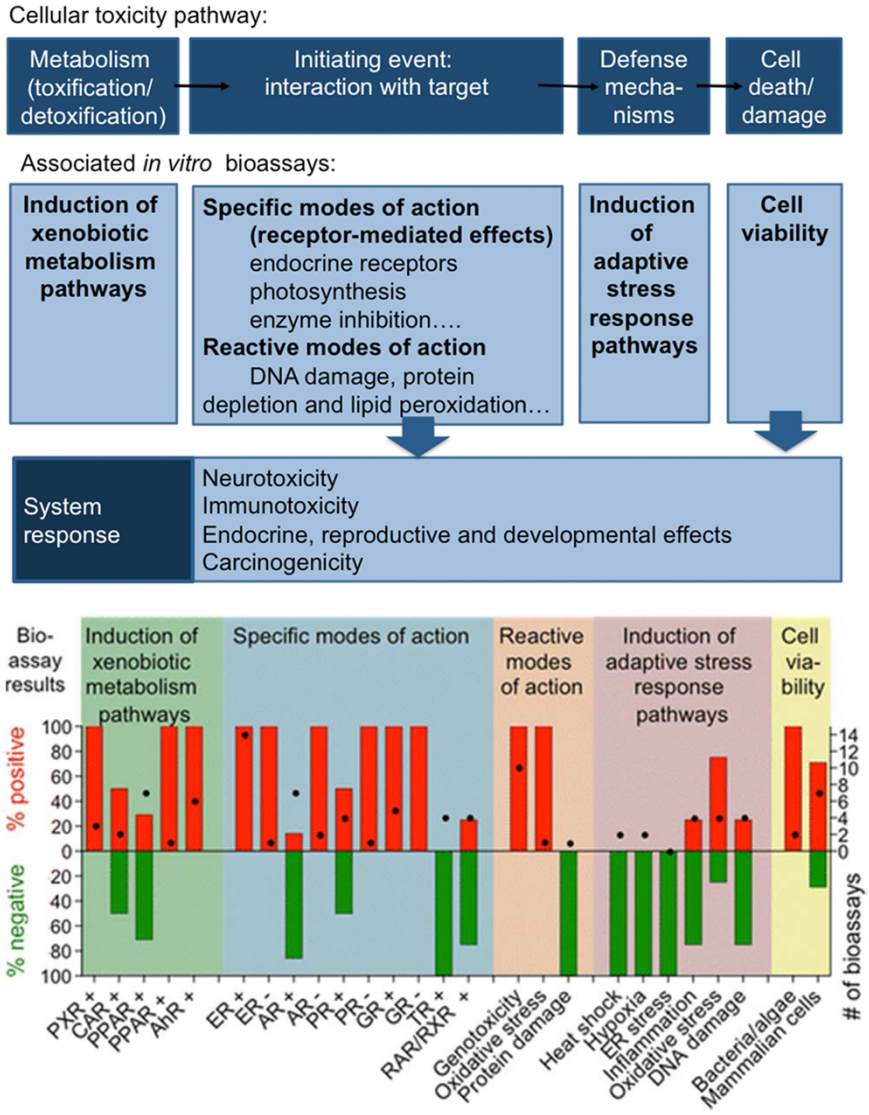


Flow Diagram for Analysis of Unknown EA Chemicals & Extracts



The BG1Luc-ER-TA bioassay was approved internationally by the OECD (TG455 and TG457) for screening for estrogenic chemicals and also by the USEPA (included in the EDSP).

Example: Combined Use of Bioassays for Water Quality Screening
(20 laboratories analyzed 10 water samples using 103 unique bioassays)



toxicity pathway	MOA	inducing chemicals/ *positive controls*	total	+	–
xenobiotic metabolism	pregnane X receptor (PXR)	steroids/	3	3	0
	constitutive androstane receptor (CAR)	phenobarbitol, various pharma-ceuticals	2 (1)	1	1
	peroxisome proliferator-activated receptor (PPAR)	phthalates, fibrate pharmaceuticals	7 (1)	2	5
	PPAR suppression		1	0	1
	aryl hydrocarbon receptor (AhR)	PAHs, PCDDs, coplanar PCBs	6 (1)	6	0
specific MOA	acetylcholinesterase (AChE)	insecticides	1	0	1
	photosystem II	herbicides	1 (1)	1	0
specific receptor-mediated MOA	estrogen receptor (ER)	human hormones and industrial chemicals (xenoestrogens), *17β-estradiol*	14 (9)	14	0
	ER suppression	*4-Hydroxy-tamoxifen*	1 (1)	1	0
	androgen receptor (AR)	*(Dihydro)-testosterone*	7 (6)	1	6
	AR suppresion	*Flutamide*	2 (1)	2	0
	progesterone receptor (PR)	*Levonorgestrel*	4 (5)	2	2
	PR suppression	*Mifepristone*	1	1	0
	glucocorticoid receptor (GR)	*Dexamethasone*	5 (6)	5	0
	GR suppression	*Mifepristone*	2	2	0
	thyroid receptor (TR)	*3,3′5-Triiodo-thyronine*	4 (1)	0	4
	RAR/RXR (Reproductive and developmental effects)	*Retinoic acid*	4	1	3
reactive modes of action	genotoxicity	*4-Nitroquinoline-N-oxide*	11 (4)	11	0
	oxidative stress	PAH, electrophilic chemicals, *t-butyl hydroquinone*	1	1	0
	protein damage	*Sea-Nine*	1	0	1
adaptive stress response pathway	heat shock response	oxygen depletion (can be caused by metals)	2	0	2
	hypoxia	tunicamycin, caplain	2	0	2
	endoplasmic reticulum stress	high salt, glycol	0	0	0
	inflammation	metals, PCBs, smoke, particles	4	1	3
	oxidative stress	reactive oxygen species, *t-butyl hydroquinone*	4	3	1

Escher et al. Env. Sci. Tech. 48, 1940 (2014)

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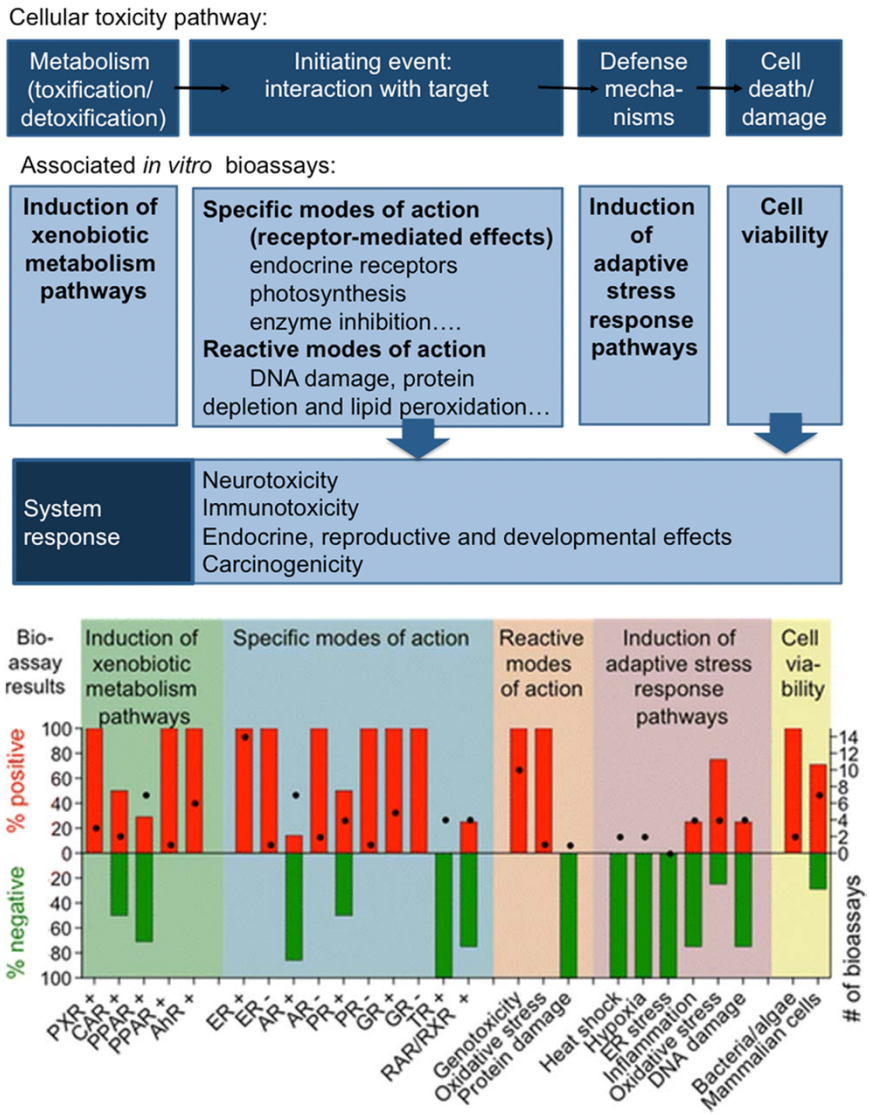


Tested: wastewater effluent, recycled water, stormwater, surface water and drinking water samples.

- Each water type had a characteristic bioanalytical profile with particular groups of “toxicity” pathways and were consistently positive or negative across test systems.

- The most responsive health-relevant endpoints were related to xenobiotic metabolism, hormone receptor pathways, genotoxicity, oxidative stress responses.

- The study demonstrated the utility of selected cell bioassays to benchmark water quality and the authors recommended a purpose-tailored panel of bioassays for routine monitoring.

Escher et al. Env. Sci. Tech. 48, 1940 (2014)

Considerations and Future for Bioassay Development and Applications for Screening/Biomonitoring Purposes

Bioassay Improvements

- Multiplexing of several mechanisms into a single cell so it is capable of detecting several classes of chemicals instead of one.
- Increased bioassay sensitivity, response and speed, lower cost.
- Bioassays made to be more relevant to the in vivo situation.

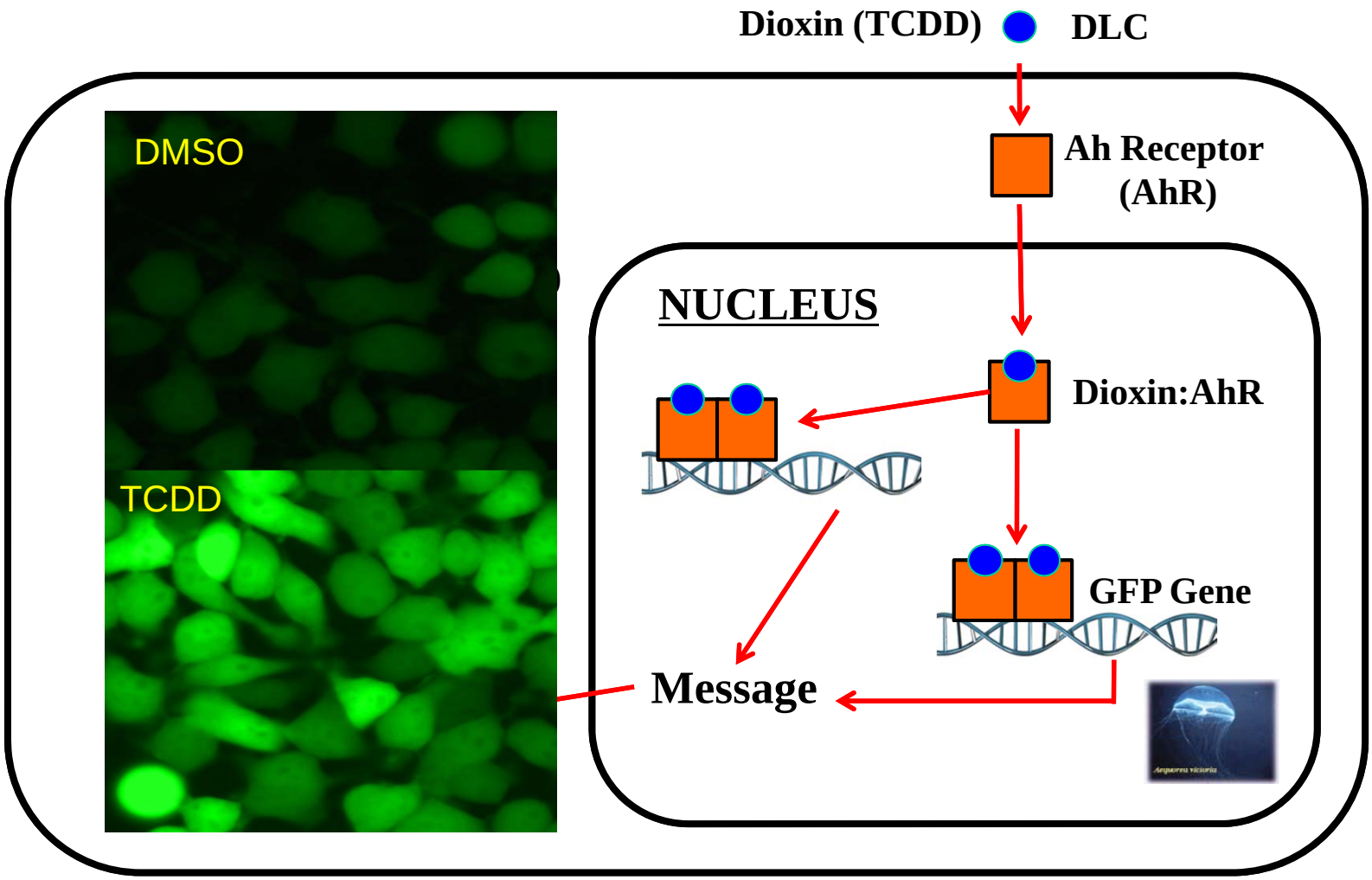
Bioassay Validation

- Appropriate validation studies need to be in place, including: double-blinded analysis and multi-laboratory validation studies.

Identify Relevant Bioassays for Monitoring Purposes

- Toxicologically relevant endpoints - what bioassays are needed?
- Need bioassays with strong relationship/relevance to AOP.
- Need critical interpretation of results. What does a positive result tell you and what does a negative result tell you… or not tell you?

Mechanism of Dioxin Action - Bioassay

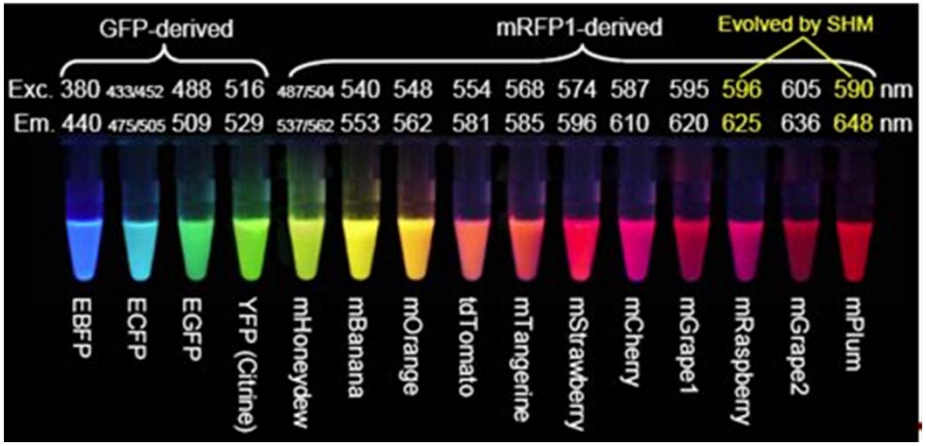


Dioxin-Responsive Cell

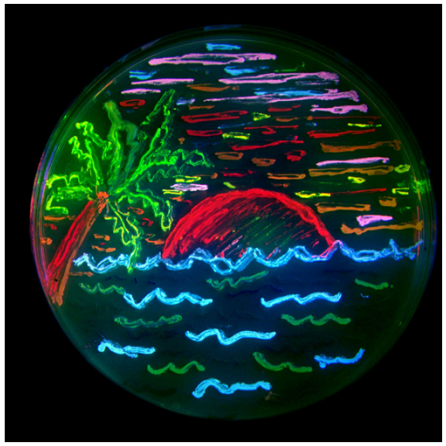
DLC – Dioxin-Like Chemical : GFP – Green Fluorescent Protein

The Availability of Multicolor Fluorescent Proteins Provide an Avenue to Multiplex Several Gene Expression Based Bioassay Systems (i.e., Hormone Receptors) in a Single Cell

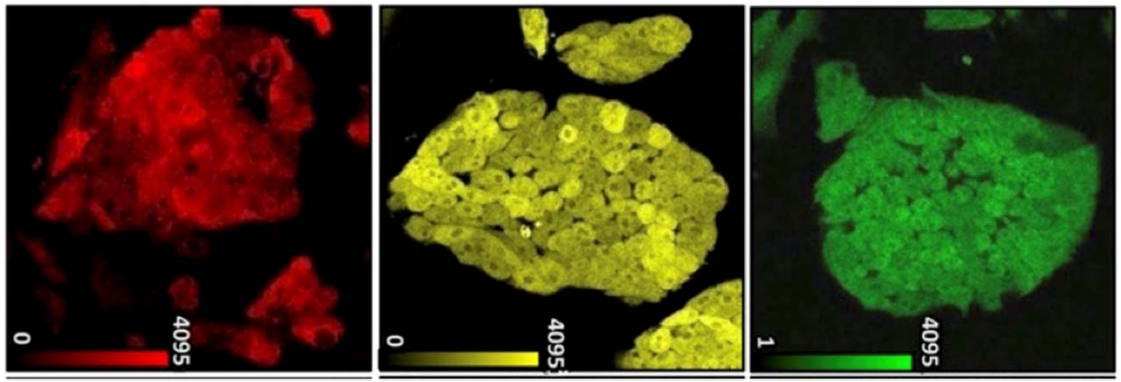
Variety of Fluorescent Proteins



Bacterial Expression

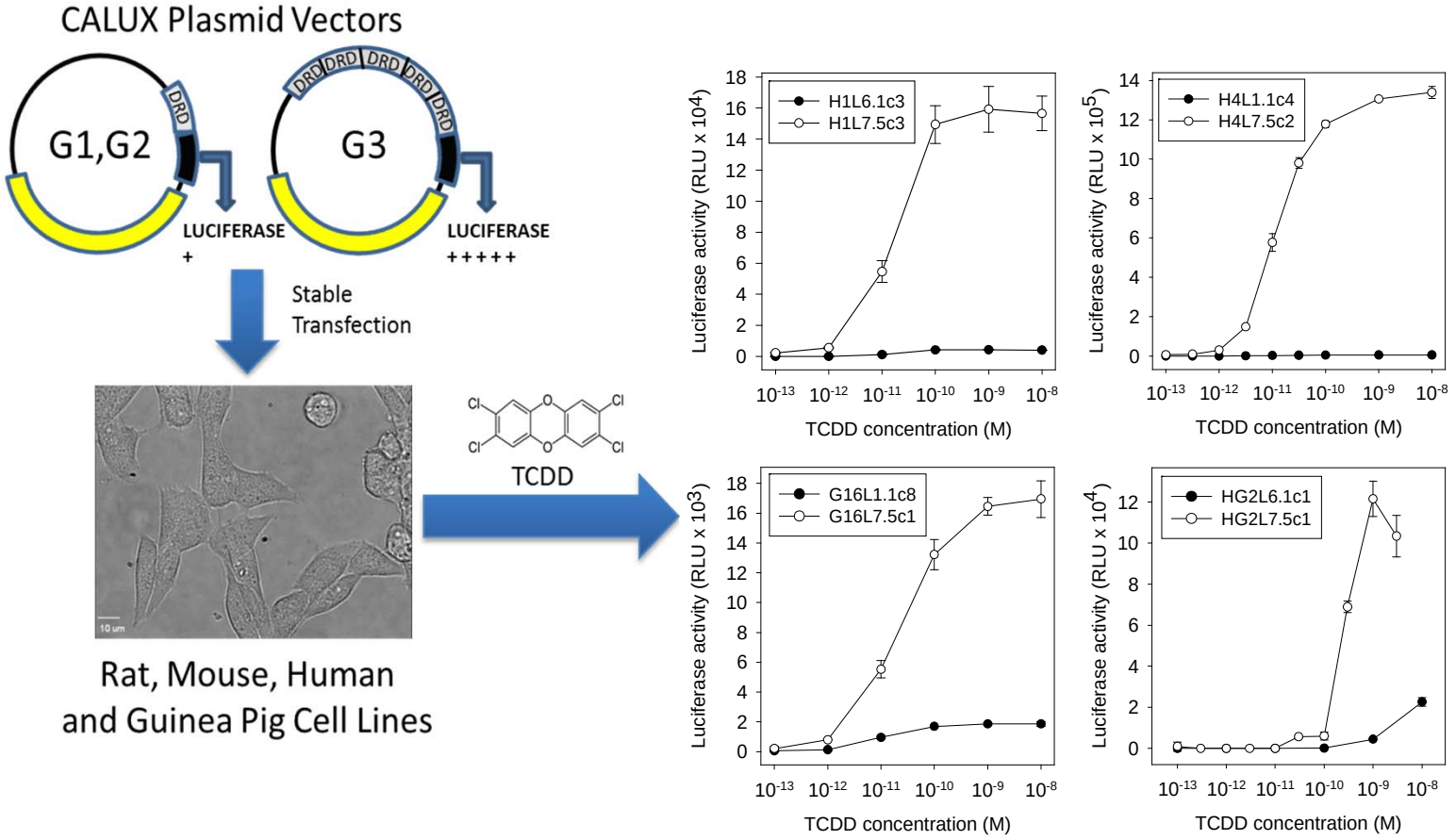


Mammalian Cell Expression



In Vivo Expression (Transgenic Fish)

Improvements in Cell Lines Can Dramatically Increase Bioassay Response and Minimal Detection Limits



Making In Vitro Cell Bioassays Closer to In Vivo Bioassays

Improved Estrogen Receptor (ER) Cell Lines for Screening: Addition of ERβ Identifies New Estrogenic Chemicals

BG1Luc4E2 (ERα) BG1LucERβc9 (ERα/ERβ)

Plate 1

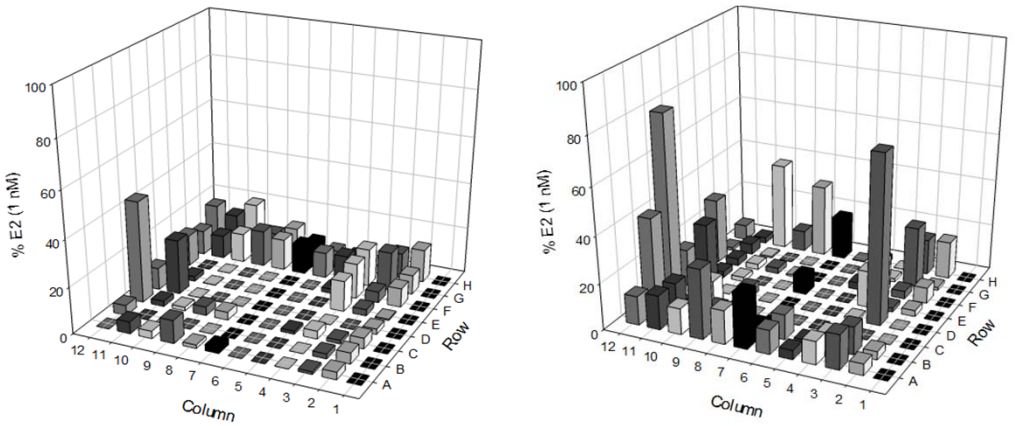
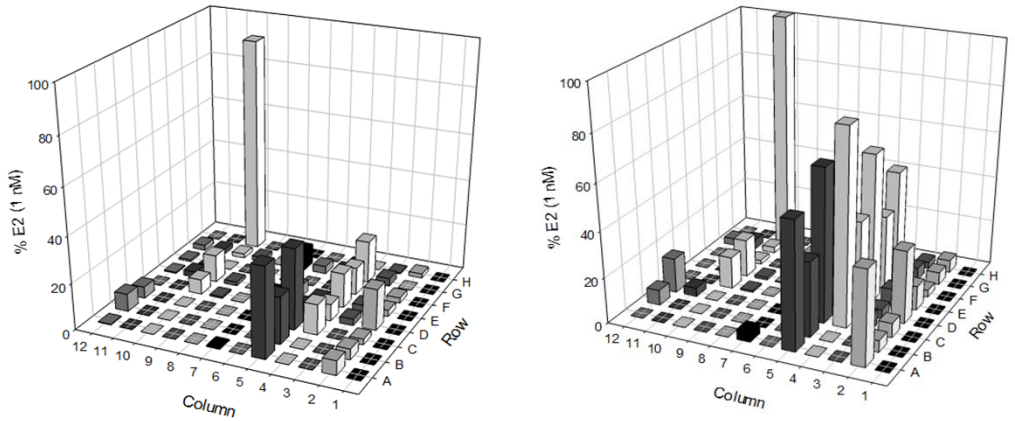


Plate 2



Chemical library: 176 chemicals ((2) 96-well plates, 10 μM test conc.)
(Pesticides, Herbicides, Fungicides, Industrial Chemicals, Drugs, Detergents, etc)

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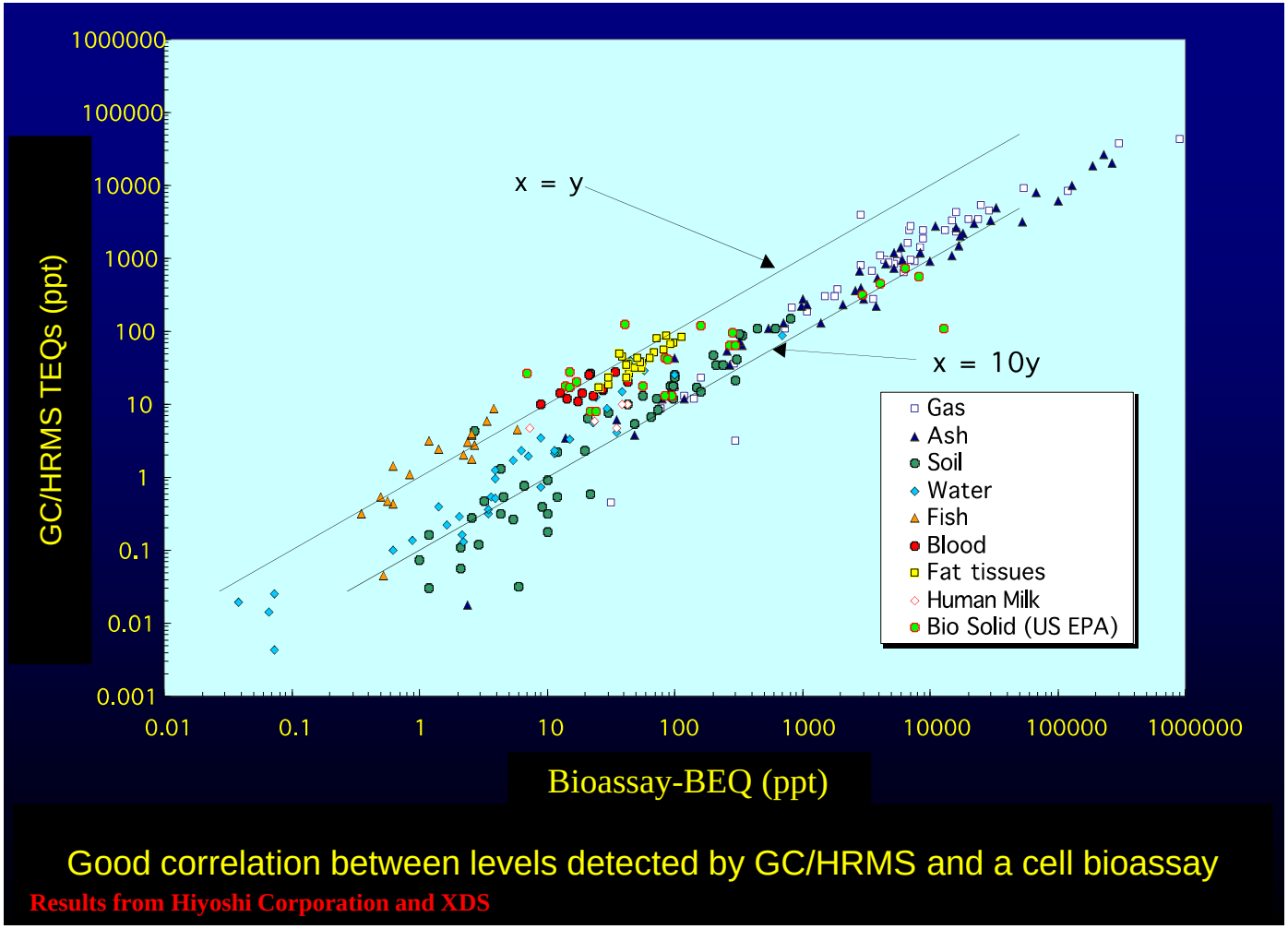
Double-Blind Validation Results for Detection of Dioxin-Like Chemicals (Comparison of Instrumental (GC/HRMS) and Cell Bioassay Analysis)



Few bioassays with sufficient or appropriate validation, cross-laboratory analysis, or regulatory certification to support their use for environmental screening purposes (depending on their application).

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Other Considerations for the Continued and Future Development and Use of Bioassays for Biomonitoring

- Coverage of chemical and biological space is incomplete:
  - Some targets = multiple orthogonal assays (e.g., estrogen receptor)
  - Some targets = one assay (e.g., thyroid receptor) or none….
- Generally bioassays have been limited to receptor-dependent effects and there are many non-receptor mediated ways of affecting an AOP.
- Many bioassays are not clearly linked to an AOP (utility for screening?).
- Bioassays are not instantaneous, but take time (most cell assays 12-24 hrs).
- Lack of metabolic capability of many cell lines (may miss active metabolites).
- Bioassays use acute chemical exposures.
- Many bioassays are proprietary technology and can be expensive.

While bioassays can not cover all biological and toxicological endpoints, neither do many in vivo non-human test systems. However, bioassays are useful for detection of chemicals affecting key steps in targeted AOPs.

Conclusions

• Bioassays provide an avenue to identify the effects of chemicals mixtures (known & unknown) on selected biological/toxicological pathways (AOPs).

• Appropriate extraction/clean-up methods can be used with selected "toxic pathway" bioassays to identify samples with in vivo toxic potential. But few clearly relevant assays are available (AhR, ER, other receptors, genotoxicity?).

• While bioassays can be used to identify specific molecular and cellular responses affected by a chemical/mixture/extract, there are considerations:

A. Toxic potential of chemical/extract in vivo? [AOP considerations]
B. Extraction method used? [polar and nonpolar chemicals]
C. Identity of chemical(s)? [unknown chemical mixtures]
D. Mixture interactive effects? [inhibition/additive/synergism]

• There remains a clear need to continue the development, validation and application of sensitive, rapid, inexpensive and validated bioassays that are closely linked to AOPs for biomonitoring and screening purposes.

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