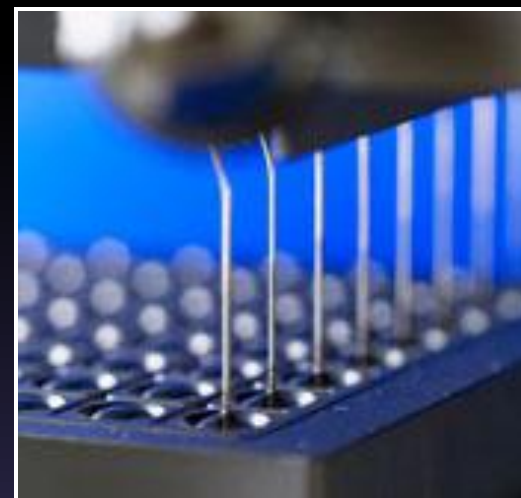


# A Strategy for Reducing Animal Use in the U.S. EPA's Endocrine Disruption Screening Program



Catherine Willett, PhD  
Science Policy Advisor



Patricia Bishop, M.S.  
Research Associate



Kristie Sullivan, MPH  
Science and Policy Advisor



# EDSP Organization

## 1. Prioritization: exposure potential

## 2. Tier 1 “screening” battery

- ER binding
- ER Transactivation
- AR binding
- Steroidogenesis
- Aromatase
- Uterotrophic
- Hershberger
- Pubertal female
- Pubertal male
- Amphibian metamorphosis
- Fish short term reproduction

“identify substances that have the potential to interact with the EAT (estrogen/androgen/thyroid) hormonal systems...”



## 3. Tier 2 battery

- Rodent 2-generation reproduction
- Avian 2-generation reproduction
- Fish life-cycle
- Invertebrate (mysid) life-cycle
- Others?

- Identify any adverse endocrine-related effects
- establish dose-response relationships

# EDSP Tier 1 tests

Screening Assays	# animals used/ test	EPA 2010 median cost est <sup>1</sup>
<i>In vitro</i>		
ER Binding – rat cytosol	?	\$17,250
ER $\alpha$ Transcriptional Activation		\$15,000
AR Binding - rat cytosol	?	\$17,250
Steroidogenesis H295R		\$13,750
Aromatase Recombinant		\$25,000
<i>In vivo</i>		
Uterotrophic	18	\$43,050
Hershberger	18 - 36	\$47,400
Pubertal Male	45	\$93,500
Pubertal Female	45	\$87,100
Fish Short-term Reproduction	72	\$92,500
Amphibian Metamorphosis	320	\$80,597
Analytical chemistry		\$12,000
<b>Total</b>	<b>518-536</b>	<b>\$544,397</b>

<sup>1</sup> Draft Guidance Document (GD) on the Assessment of Chemicals for Endocrine Disruption (version 9), 2010. Organization for Economic Cooperation and Development (OECD).

# EDSP Phase I Chemicals

67 chemicals

- 58 pesticide active ingredients (PAIs)
- Nine High Production Volume (HPV) chemicals pesticide inerts
- Most have a wealth of existing data, e.g. pesticide registration:
  - metabolism and pharmacokinetics studies
  - reproductive/lifecycle studies in rodents, fish and birds
- These studies include many of the endpoints addressed in EDSP Tier 2 tests

If all tests were done would require > 35,000 animals & > 36 million USD

EDSP: cost / benefit?

# OMB Terms of Clearance

EPA should promote and encourage test order recipients to submit Other Scientifically Relevant Information (OSRI) in lieu of performing all or some of the Tier I assays, and

EPA should accept OSRI as sufficient to satisfy the test orders to the greatest extent possible.

# EPA' s definition of OSRI

- informs the determination as to whether the substance may have an effect that is similar to an effect produced by a substance that interacts with the E/A/T hormonal systems
- (is) **functionally equivalent** to information obtained from Tier 1 assays
- provides information on **a potential consequence** or effect that could be due to effects on the E/A/T systems.
- testing conducted under 40 CFR Part 158 data requirements to support a pesticide registration may be considered by EPA

# EDSP Phase I Use of OSRI†

Tier 1 Assay	OSRI Proposed	Assay Proposed	OSRI Accepted by EPA	OSRI Denied by EPA	# Animals in Proposals	# Animals used after EPA Review
ER Binding	45	2	10 (22%)	35	-	-
AR Binding	45	2	9 (20%)	36	-	-
ERTA	44	3	11 (25%)	33	-	-
Aromatase	37	10	5 (14%)	32	-	-
Steroidogenesis	30	17	6 (20%)	24	-	-
Male Pubertal	44	3	12 (27%)	32	135	1,575
Female Pubertal	43	4	8 (19%)	35	180	1,755
Hershberger	31	16	12 (39%)	20	288 - 576	10,368 - 20,736
Uterotrophic	35	12	11 (31%)*	24	216	648
Fish Short-term	26	21	1 (4%)	25	1,512	3,312
AMA	32	15	4 (13%)	28	4,800	9,600
<b>Total</b>	<b>412</b>	<b>105</b>	<b>89 (22)</b>	<b>323</b>	<b>7,131 - 7,419</b>	<b>27,258 - 37,626</b>

†Results from 47 chemicals reviewed by EPA to date

# EPA Reasons for Rejection of OSRI

## **Blanket rejection of ToxCast data:**

–ToxCast was designed to prioritize chemicals

## **Part 158 developmental and multi-generation reproductive testing information:**

–Protocols do not address mechanism of action (!)

## **Nearly universal rejection of fish studies to satisfy FSTR Assay:**

–Lack of measurement of egg number or gonadal histopathology in reproductive and life-cycle studies

## **Slight deviations from OPPT 890 protocols:**

–Hershberger: use of intact vs castrate males (a variation of OPPT 890.1400)

–Assessed thyroid histopathology but did not use 5-point grading system

–In reproductive tests estrous cyclicity or time to vaginal opening was not measured

# Conclusions Regarding OSRI use in Phase I

Existing information was used primarily in cases where it was identical to the information that would be obtained by performing OPPTS series 890 protocols

Data indicating a positive response was accepted preferentially over negative data (*in vitro* or *in vivo*).

OSRI were treated inconsistently among chemicals

OSRI had minimal impact on animal use in EDSP Phase I

This type of complex, animal-intensive testing is inappropriate as a general screen for chemicals that may possibly interact with the E/A/T pathways

# A more efficient strategy is needed

- Initial assessment takes into account all existing information including physical, chemical, exposure, QSAR estimates
- Secondary screen includes all available mechanistic assays, etc.
- Define positive and negative cut-offs at the outset for each test and each Tier
- Results are used to design the strategy for further testing
- Tests are carefully designed to address information needs
- Sufficient regulatory information is obtained while limiting animal testing and use of resources

# Based on OECD Conceptual Framework

**Level 1      *Sorting and prioritization based on existing information***

- physical and chemical properties
- exposure
- available toxicological data

**Level 2      *In vitro assays providing mechanistic data***

- QSARs
- binding and transcription assays
- aromatase, steroidogenesis, thyroid function
- high-throughput screens

**Level 3      *In vivo assays addressing single mechanisms and effects***

- uterotrophic
- Hershberger
- amphibian metamorphosis
- fish VTG

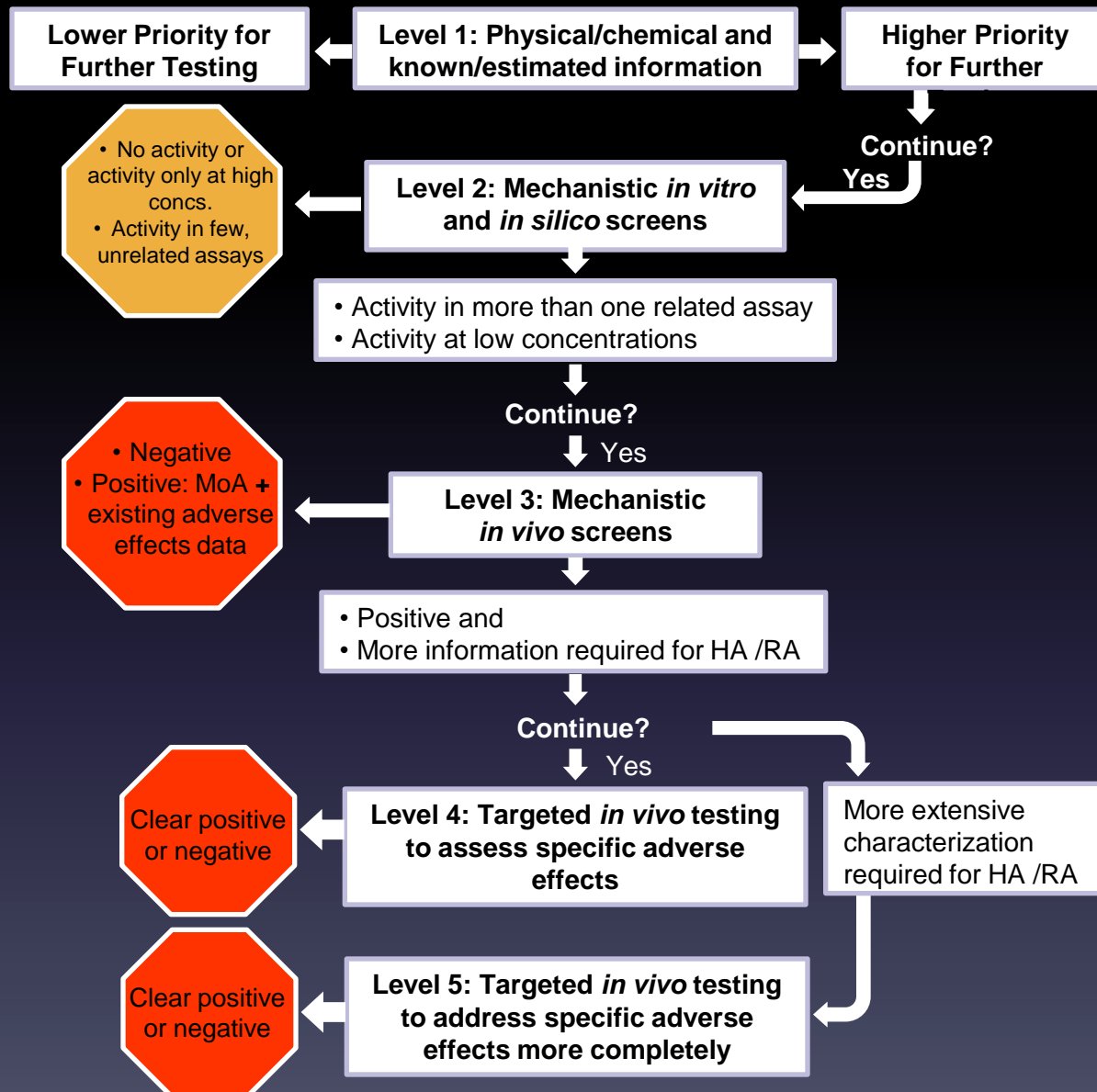
**Level 4      *In vivo assays addressing multiple mechanisms and effects***

- enhanced 407
- male and female pubertal assays
- adult intact male
- fish short-term reproductive assay

**Level 5      *In vivo assays providing data on effects from endocrine + other mechanisms***

- 1 and 2 generation reproduction in rodents, birds, fish

# Refined Tiers + Iterative Evaluation



# Reference

Willett, C., P. Bishop and K. Sullivan.

Application of an Integrated Testing Strategy to the U.S. EPA Endocrine Disruptor Screening Program.

Toxicological Sciences online – June 3, 2011

Paper journal – coming in September.

# Thank you.

Catherine Willett, PhD  
*Science Policy Advisor and  
Associate Director  
Regulatory Testing Division  
People for the Ethical Treatment of Animals  
T: 617-522-3487  
katew@peta.org*

