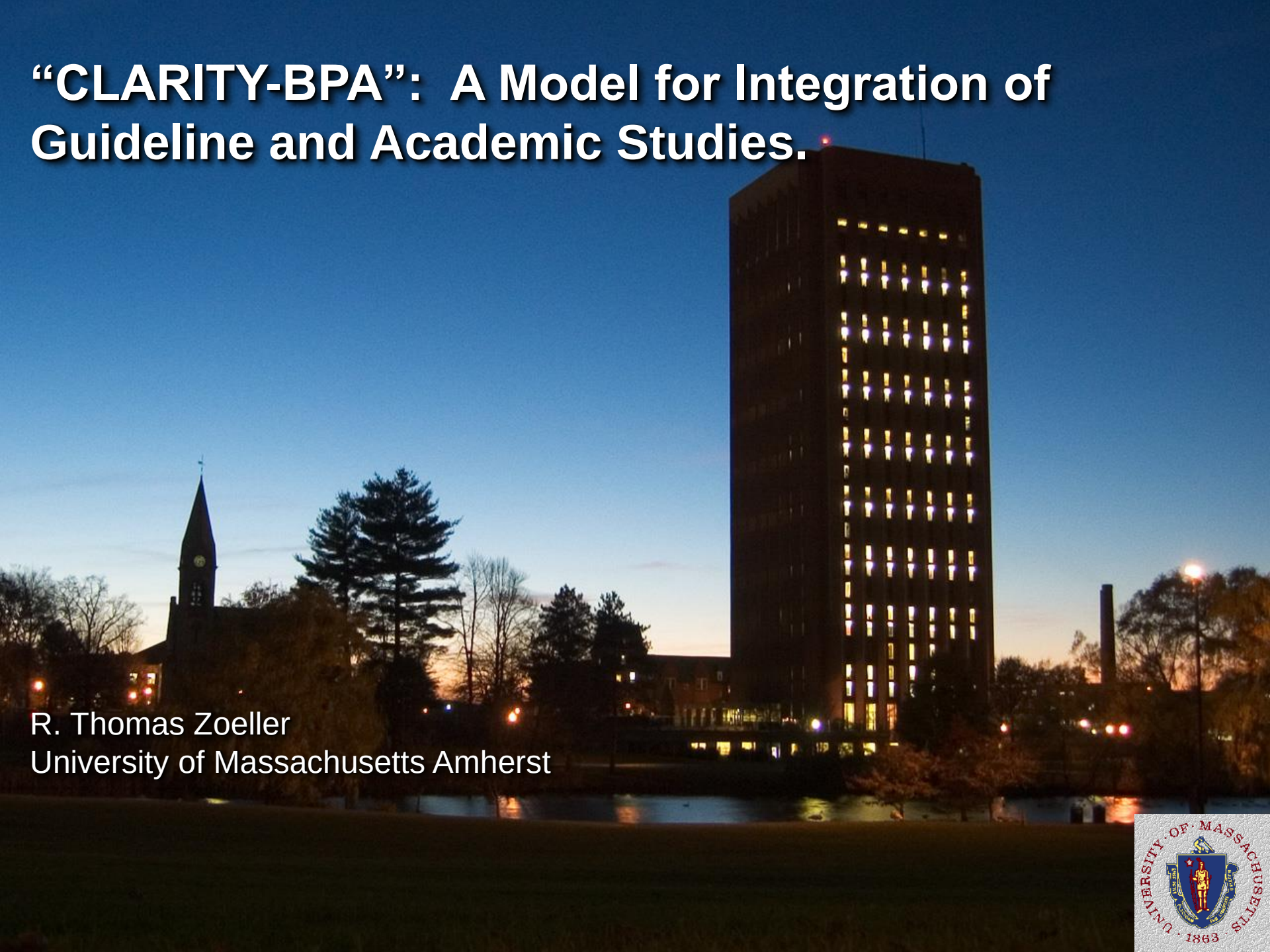
“CLARITY-BPA”: A Model for Integration of Guideline and Academic Studies.



R. Thomas Zoeller
University of Massachusetts Amherst

Disclaimer

The information conveyed in this presentation does not necessarily reflect the opinion of the FDA, the National Institute of Environmental Health Sciences (NIEHS), or the National Toxicology Program (NTP).

CLARITY-BPA

- Consortium Linking Academic and Regulatory Insights on the Toxicity of BPA
- Research consortium involving scientists from:
  - FDA/NCTR and CFSAN
  - NIEHS/NTP and DERT (Division of Extramural Research/Training)
  - 13 NIEHS-funded university-based grantees
    - Selected through a NIH competitive grant review process
    - FDA/NCTR assessed applications for feasibility only
- Goal was to integrate data from university-based grantee studies with those of a guideline-compliant study to contribute to the safety assessment of BPA

CLARITY-BPA

Why?

Arch Toxicol
DOI 10.1007/s00204-016-1866-9

MEETING REPORTS

Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement

Roland Solecki[1] · Andreas Kortenkamp[2] · Åke Bergman[3] · Ibrahim Chahoud[4] · Gisela H. Degen[5] · Daniel Dietrich[6] · Helmut Greim[7] · Helen Håkansson[8] · Ulla Hass[9] · Trine Husoy[10] · Miriam Jacobs[11] · Susan Jobling[2] · Alberto Mantovani[12] · Philip Marx-Stoelting[1] · Aldert Piersma[13] · Vera Ritz[1] · Remy Slama[14] · Ralf Stahlmann[4] · Martin van den Berg[15] · R. Thomas Zoeller[16] · Alan R. Boobis[17]

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22. We agree that a chemical's potency to induce an adverse effect is an important factor for consideration during the characterization of the hazards of endocrine disruptors. However, potency is not relevant for identification of a compound as an endocrine disruptor.

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11. We acknowledge that certain hormones interact with their receptors according to an equilibrium reaction. Accordingly, the concentrations of both free hormone and free receptor are important variables controlling hormone action, explaining why different cells and tissues at different times during development are differentially sensitive to the hormone.

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16. On the other hand, the existing framework of internationally validated test systems for the identification of endocrine disruptors must be further developed to ensure detection of health effects relevant to endocrine disruption in humans. For example, test systems

CLARITY-BPA
Why?

- Estrogen controls many different events during development and in adulthood
- Guideline endpoints do not fully capture all these endpoints
- Different endpoints will be differentially-sensitive to BPA
- Thus, hazard characterization may be inappropriate.

CLARITY-BPA *Design?*

Core Study at NCTR
Guideline-GLP Compliant

↓

Full battery of guideline assays

↓

Tissues sent to academic labs for specific analyses

NCTR 2-YEAR CHRONIC (CLARITY-BPA "CORE") STUDY

- Modified guideline, GLP-compliant study
- All CLARITY-BPA stakeholders contributed to the final design of the core study
  - Face-to-face meeting at FDA/NCTR
- Animal model: NCTR Sprague-Dawley rat

Study Design (continued)

- 50 animals per dose group, per sex, per age in core study
- Additional animals provided for grantee studies (~10/dose gp)
- Potential sources of background BPA including diet, cages (polysulfone), bedding, drinking water, and water bottle stoppers were measured and monitored.
- Animals were fed a diet free in soy and alfalfa. It was certified to have <2 ppm genistein and daidzein, <0.5ppm coumestrol and zearalenone and <5 ppm BPA.
- Limited internal dosimetry measurements : all BPA and EE2 doses.
- In summary, all aspects of the CLARITY-BPA study followed a shared, robust protocol for animal housing and feeding, compound handling and dosing, measurements and evaluation, and sharing and storing of samples.

NCTR 2-YEAR CHRONIC (CLARITY-BPA "CORE") STUDY (cont.)

- Dose groups:
  - BPA: 2.5, 25, 250, 2,500, and 25,000 µg/kg bw/day
  - Ethinyl estradiol: 0.05 and 0.5 µg/kg bw/day (reference estrogen)
  - Vehicle control (0.3% carboxymethylcellulose in water)
- Daily oral gavage, 7 days a week
- Direct dosing of the F_1 pups from the day after birth
- Two dosing arms:
  - "Continuous dose" arm: from gestation day 6 through termination
  - "Stop dose" arm: from gestation day 6 through weaning (PND21)

CLARITY-BPA Grantees and Areas of Study

Principal Investigator	Disease Focus	Endpoint	Aims Funded
Heather Patisaul/ Cheryl Rosenfeld	**Learning and behavior** *stop dosing only*	Brain transcriptomics *birth* Behavioral *PND 21 and 90*	1) brain gene expression 2) behavioral assess. pnd 21 3) behavioral assess. pnd 90
Ana Soto	**Breast cancer**	Breast development and cancer *PND 21 and 90; 6 months (whole mounts)*	1)breast morphology at pnd 21 as precursor of cancer 2)gene expression and DNA methylation at pnd 21 3)assess pre-neoplastic lesions and neoplastic lesions pnd 90 and 6 mo.
Gail Prins	**Prostate cancer**	Prostate gene expression, and cancer development *PND 21; 6, 12 months*	1) prostate gene expression 2) prostate methylation 3) renewal of stem cells 4) assess PIN and cancer

Principal Investigator	Disease Focus	Endpoint	Aims Funded
Shuk Mei Ho	**Uterine cancer** Continuous dosing only	Uterus histology and gene expression *6, 12 months*	1) histological ID of uterine hyperplasia/adenocarcinoma 2) laser capture to assess methylome and transcriptome to identify early cancer genes
Fred vom Saal	**Male urogenital abnormalities**	Urogenital system analysis *Birth; 12 months*	1) 3-D reconstruction of urogenital system 2) examine animals for voiding and laser capture to assess gene expression in epithelium and stroma
Nestor Gonzalez-Cadavid	**Penile function**	Penile erection mechanism *6 months*	1) erection capability, transcriptomic profile and stem cell analysis

Principal Investigator	Disease Focus	Endpoint	Aims Funded
Kim Boekelheide	**Testis function/ sperm counts** Continuous dosing only	Testis and epididymis *PND 90; 12 months*	1) histological and morphological assessment of testis 2) caudal sperm transcriptome 3) caudal sperm methylome
Jodi Flaws	**Ovarian function**	Ovary Birth; pnd *21, 90; and 12 months*	1) follicle number 2) steroidogenic enzymes
Andrew Greenberg	**Diabetes, blood glucose, pancreas, liver**	Blood glucose and pancreas assessment 12 months	1) assess blood glucose over time and beta cell mass and insulin content 2) liver histopathology

Principal Investigator	Disease Focus	Endpoint	Aims Funded
Nira Ben Jonathan	**Obesity/adipose tissue**	adipose tissue disposition and weight gain *PND 90; 6 and 12 months*	1) fat depots and selected adipokines, gene expression 2) serum hormones 3) adipose cell number & size
Norbert Kaminski	**Immune Function** *continuous dosing only*	Spleens assessed *PND 21, 90; 6 and 12 months*	1) spleen T & B cell subpopulations 2) response to stimulation 3) ER characterization 4) gene expression
Tom Zoeller	**Thyroid and brain anatomy**	Thyroid and brain development *PND 15*	1) changes in brain gene expression and histology due to BPA impact on thyroid hormones
Scott Belcher	**Cardiovascular**	Heart *PND 21, 6 mo*	1)heart structure 2)ventricular wall thickness and fibrosis 3) cardiac hypertrophy

RESULTS

- **1.** Arambula SE, Belcher SM, Planchart A, Turner SD, Patisaul HB. Impact of Low Dose Oral Exposure to Bisphenol A (BPA) on the Neonatal Rat Hypothalamic and Hippocampal Transcriptome: A CLARITY-BPA Consortium Study. *Endocrinology.* 2016;157(10):3856-3872.

- 2. SA, Javurek AB, Painter MS, Ellersieck MR, Welsh TH, Jr., Camacho L, Lewis SM, Vanlandingham MM, Ferguson SA, Rosenfeld CS. Effects of developmental exposure to bisphenol A on spatial navigational learning and memory in rats: A CLARITY-BPA study. *Horm Behav.* 2016;80:139-148.

- **3.** Heindel JJ, Newbold RR, Bucher JR, Camacho L, Delclos KB, Lewis SM, Vanlandingham M, Churchwell MI, Twaddle NC, McLellen M, Chidambaram M, Bryant M, Woodling K, Gamboa da Costa G, Ferguson SA, Flaws J, Howard PC, Walker NJ, Zoeller RT, Fostel J, Favaro C, Schug TT. NIEHS/FDA CLARITY-BPA research program update. *Reprod Toxicol*. 2015;58:33-44.

- **4.** Rebuli ME, Camacho L, Adonay ME, Reif DM, Aylor DL, Patisaul HB. Impact of Low-Dose Oral Exposure to Bisphenol A (BPA) on Juvenile and Adult Rat Exploratory and Anxiety Behavior: A CLARITY-BPA Consortium Study. *Toxicol Sci*. 2015;148(2):341-354.

- **5.** Schug TT, Heindel JJ, Camacho L, Delclos KB, Howard P, Johnson AF, Aungst J, Keefe D, Newbold R, Walker NJ, Thomas Zoeller R, Bucher JR. A new approach to synergize academic and guideline-compliant research: the CLARITY-BPA research program. *Reprod Toxicol*. 2013;40:35-40.

THOUGHTS FOR POSSIBLE FUTURE PROJECTS

- Important to recognize that this is a first-ever collaboration and we need to learn from it.
- Greater development and organization of goals and strategies prior to RFA (call) could streamline the project and improve utility.
- Early, detailed description of the animal/sample collection requirements by the university researchers would provide a better assessment of the feasibility of the studies and help streamline the “core” study

THOUGHTS FOR POSSIBLE FUTURE PROJECTS

- The collaborative design could also be employed to identify new endpoints to be included into guideline studies
  - For example, tissues derived from the core study could be sent to academic labs for analysis. Blinded samples sent to triplicate labs could simultaneously evaluate those endpoints, but validate their reliability.

THOUGHTS FOR POSSIBLE FUTURE PROJECTS

Core Study
Guideline-GLP Compliant

↓

Full battery of guideline assays

↓

Tissues sent to
3 different academic labs for the same specific analyses

THANK YOU FOR YOUR ATTENTION!!