SETAC NORTH AMERICA FOCUSED TOPIC MEETING

ENDOCRINE DISRUPTION

Chemical Testing, Risk Assessment Approaches and Implications

MEETING PROGRAM

4–6 FEBRUARY 2014 | USEPA RESEARCH TRIANGLE PARK, NC | ENDOCRINE.SETAC.ORG

Welcome!

Welcome to the SETAC North America Focused Topic Meeting on Endocrine Disruption: Chemical Testing and Risk Assessment Approaches and Implications!

The Meeting Steering Committee, headed up by co-chairs Annegaaike Leopold and Holly Zahner, has done an excellent job in creating a compelling two-day program that covers global developments and advancements on testing and risk assessment approaches for endocrine disrupting chemicals in the environment. The program includes engaging presentations and invaluable opportunities to network with leading scientists working on these topics. My thanks also goes to the entire SETAC North America staff for helping to organize the myriad of meeting details, but especially to Nikki Mayo, Terresa Daugherty, Jason Andersen, Sabine Barrett and Bruce Vigon, who were instrumental in making this meeting happen.

If you are a presenter at this meeting, please consider publishing your research in one of the SETAC journals—now without page charges for authors! For the future, we hope to build on the success of this meeting by offering similar meetings on a more frequent basis. So I encourage you to provide your feedback through the post-meeting survey, especially regarding topics that you would find of interest. And finally, if you are not a current SETAC member, please consider joining our outstanding Society.

Executive Director
SETAC North America

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Annegaaike Leopold

A special thank you to the Meeting Co-Chairs!

Holly M. Zahner

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THANK YOU!

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Meeting Policies

Code of Conduct

Members of the Society of Environmental Toxicology and Chemistry are expected to exhibit the highest standards of integrity and professionalism. To ensure a strong and successful organization, our activities require honesty and equity and should reflect well on the Society. In the spirit of promoting Environmental Quality through Science®, members should strive to be good stewards of environmental resources and effective and objective contributors to the environmental discussion globally as well as locally.

Professional behavior and integrity are also expected from every attendee (members and nonmembers alike) of SETAC meetings, workshops and activities.

Each member and all persons participating in SETAC meetings and activities are bound by this code of ethics and should:

- Conduct themselves responsibly, objectively, lawfully and in a nondiscriminatory manner
- Ensure that presentations during Society-sponsored events and other communications are restricted to and based on scientific principles and made in a respectful manner
- Respect the rights, interests and contributions of professional colleagues
- Respect intellectual property and provide appropriate attribution for all intellectual property arising elsewhere
- Declare and avoid conflicts of interest
- Not knowingly make false or misleading statements, or engage in activities that could be viewed as defamatory about a professional colleague or an organization
- Recognize and respect confidentiality while being honest and forthcoming in all issues of public record
- Objectively and clearly communicate scientific methods, understanding and knowledge in a professional manner
- Conduct research and related activities so as to avoid or minimize adverse environmental effects of that research, and ensure compliance with legal requirements for protection of researchers, human subjects, and research organisms and systems

Cell Phones

Out of courtesy to our speakers and attendees, we require that all cell phones be turned off during sessions and meetings.

Environmental Awareness

In maintaining a high standard of environmental awareness and compliance, this meeting is designated nonsmoking. To the best of our ability, recycled and recyclable food and beverage containers will be used, with segregated refuse containers readily available.

Media Policy

Media representatives are cordially invited to attend SETAC North America focused topic meetings.

SETAC's media policy is designed to ensure a professional forum in which presenters and other meeting registrants can discuss science-based issues freely and in which their concerns about proprietary research data and other information is acknowledged and respected. It is further designed to ensure a forum in which journalists and other media representatives can gather the information they need to deliver factual reporting.

SETAC can provide:

- Press releases and news updates
- Photos, videos or other supporting files when available
- Assistance arranging interviews with SETAC officers, members and presenters before or at the meeting

In return, we expect you to:

- Wear your media badge, and identify yourself as a member of the press when you attend presentations or talk with any group or individual
- Obtain permission from the SETAC North America Executive Director, Greg Schiefer, and from the subject presenters or meeting registrants before you film, tape or otherwise record any activity or interview at this event
- Respect a presenter's or meeting participant's choice to talk with you or to decline

Pre-registration is required for all attendees of the SETAC North America Focused Topic Meeting on Endocrine Disruption.

Any media representative who sells, markets or represents a company for purposes of obtaining advertising or subscriptions from any exhibitor or registrant will immediately forfeit press credentials for this and subsequent meetings.

Audio, Photo and Video Policy

Recording, Photographing, Interviewing Not Allowed

No attendee at a SETAC annual meeting may record, film, tape, photograph, interview or use any such media during any presentation, poster display or exhibit without the express, advance approval of the executive director of SETAC North America. This policy applies to all SETAC members, nonmembers, guests and exhibitors as well as members of the print, online or broadcast media.

Photo Release

Photographs will be taken at the SETAC North America Focused Topic Meeting on Endocrine Disruption. By registering for this meeting, you agree to allow SETAC to use your photo in any SETAC-related publication or website.

Facility Policy

Please remember that all conference attendees are required to stay in the meeting space. This includes the session room, poster area, atrium, restrooms and café.

Exhibitor Information

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Established in 1968, ABC is a privately held company of about 400 scientists and support personnel. We provide a broad array of GLP and CGMP-compliant product development and analytical testing services to the agriculture and chemical industries. Our approach to doing business promotes relationships beyond a simple transaction.

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Abraxis is a biotechnology company that develops, manufactures and distributes real time detection solutions to the water quality, wastewater, and remediation markets. Product offerings include ELISA kits/systems for cyanotoxins, chemicals of emerging concern (CEC), endocrine disrupting chemicals (EDC), pharmaceuticals and personal care products (PPCP), human activity bio-markers, toxic organics, surfactants and pesticides.

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Experimental Pathology Laboratories, Inc. (EPL)

Paul Sanders
P.O. Box 12766 | Research Triangle Park, NC 27709 USA
T 919 998 9407 | **E** psanders@epl-inc.com

Experimental Pathology Laboratories, Inc. (EPL) is a GLP laboratory specializing in the collection, histologic processing, and pathologic evaluation of tissues from fish, amphibians, and aquatic invertebrates. We are experienced with the evaluation of endocrine disruptor assays and are able to customize our services to meet our clients' specific scientific, regulatory and management objectives.

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Join 2000 participants from academia, business, government and non-governmental organisations for the SETAC Europe 24th Annual Meeting in Basel, Switzerland from 11-15 May 2014. Find parallel platform and poster presentations, short courses, workshops, social events, exhibits and more.

Main tracks:

- Aquatic and terrestrial ecotoxicology
- Landscape ecotoxicology and management, ecosystem services
- Environmental and analytical chemistry
- Emerging contaminants and effects
- Fate and effects of nanomaterials
- Exposure, bioavailability and bioaccumulation
- Effect modelling and predictive toxicology
- Life cycle analysis and sustainability
- Risk assessment, regulation and public perception
- Special sessions
- Science slam NEW

Important deadlines:

Grant applications	20 February
Early registration	15 March
Online registration	15 April

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Event Hours

	Registration	Technical Program	Exhibits
Tuesday	---	1:00 p.m.–5:00 p.m.	---
Wednesday	7:30 a.m.–6:30 p.m.	8:30 a.m.–5:30 p.m.	8:30 a.m.–6:30 p.m.
Thursday	7:30 a.m.–3:00 p.m.	8:30 a.m.–4:00 p.m.	8:30 a.m.–4:00 p.m.

Poster Hours

	Setup/ Take Down	Viewing Hours	Poster Social
Wednesday	7:30 a.m.–8:00 a.m.	8:00 a.m.–6:30 p.m.*	5:30 p.m.–6:30 p.m.
Thursday	4:00 p.m.–4:30 p.m.	8:00 a.m.–4:00 p.m.*	----

* Posters must remain displayed on both days for the entire session.

Breaks

	Morning	Lunch	Afternoon
Wednesday	10:15 a.m.–10:35 a.m.	11:20 a.m.–12:30 p.m. with speaker	3:20 p.m.–3:40 p.m
Thursday	10:00 a.m.–10:15 a.m.	12:25 p.m.–1:15 p.m. on your own	2:15 p.m.–2:40 p.m.

Helpful Tips for Presenters

Are You Giving a Poster Presentation?

All poster material should be confined to the size of the poster board provided.

One author of each poster is responsible for the proper assembly, mounting and presentation of his/her poster.

Presenters will be allowed to begin mounting their display on their assigned board at 7:30 a.m.

Posters must be removed immediately at the end of the meeting.

The poster board surface consists of fabric over cork board. Push pins or Velcro can be used to mount the poster, with pushpins being the preferred method.

SETAC will provide push pins for hanging posters.

A program committee member or staff person will be present in the Poster Area and available to provide assistance and answer any questions.

Venue and Transportation

Event Address

US Environmental Protection Agency Campus

109 T.W. Alexander Drive
Research Triangle Park, NC 27711
+1 (919) 541–2258

Please remember it is USEPA Campus policy that all conference attendees are required to stay in the meeting space. This includes the session room, poster area, atrium, restrooms and café.

Conference Hotel

Fairfield Inn & Suites Raleigh-Durham Airport/Brier Creek

10040 Sellona St.
Raleigh, NC 27617
+1 (919) 246–0107

Transportation

Shuttle Information

Raleigh-Durham International Airport (RDU) is the closest airport to the USEPA Campus. If you are staying the conference hotel, there is a complimentary shuttle to and from the airport to the hotel. You can call the hotel at +1 (919) 234-4946 to arrange for the shuttle to pick you up.

If you are staying the conference hotel, Fairfield Inn & Suites Raleigh-Durham Airport/Brier Creek, there is a complimentary shuttle from the hotel to the meeting site:

- Wednesday, 5 February
  Continuously between 7:00 a.m.–9:00 a.m. and 5:30 p.m.–6:30 p.m.
- Thursday, 6 February
  Continuously between 7:00 a.m.–9:00 a.m. and 4:00 p.m.–6:00 p.m.

Taxi Information

If you need taxi service from the USEPA facility, please call to schedule at least 1–2 hours prior to pick up time.

- All American Taxi, +1 (919) 349–5038
- Taxi Taxi, +1 (919) 333–3333
- Cardinal Cab, +1 (919) 828–3228

Tuesday 4 February

Daily Schedule

Time	Event	Location
1:00 p.m.–5:00 p.m.	Professional Training Courses	Fairfield Inn & Suites
7:00 p.m.–9:00 p.m.	Opening Reception (pre-registration required)	Fairfield Inn & Suites

Professional Training Courses

The Endocrine System: Global Perspectives on Testing Methods and Evaluation of Endocrine Activity

Lead Instructor: Ellen Mihaich, Environmental and Regulatory Resources

In response to concerns that certain environmental chemicals could be interfering with the endocrine system of humans and wildlife, regulations have been promulgated in various regulatory bodies around the world to target the evaluation of these types of effects. The purpose of this professional training course is to address key topics related to endocrine system evaluation and regulatory requirements around the world. The course will provide basic information on the vertebrate endocrine system, mechanisms of control and adverse effects. The focus of the endocrine system presentation will be the estrogen, androgen and thyroid systems. The requirements of the USEPA Endocrine Disruptor Screening Program, as well as those for REACH and other regulatory initiatives around the world including the development of definitions and criteria in the EU, will be reviewed. Specific screens and tests used in these programs will be discussed, including plans for the evolution of the USEPA program such as EDSP21 and the development of adverse outcome pathways. Use of weight of evidence evaluations in interpreting the data will be covered. Finally, an interactive simulation will be staged where small groups of participants can engage in a transparent and quantitative weight of evidence evaluation of data.

Opening Ceremony

Join us from 7:00 p.m.–9:00 p.m. for drinks and hors d'oeuvres at the Fairfield Inn & Suites Raleigh-Durham Airport/Brier Creek. Pre-registration is required.

Conference Banquet

WEDNESDAY NIGHT

SOLD OUT

Join us for a sit-down dinner from 7:30-9:30 p.m. on **Wednesday night** at MEZ Restaurant, North Carolina's first LEED-certified restaurant. Guest speaker is David Berube from North Carolina State University. Tickets are $40 each and must be purchased in advanced. Two drinks are included. Transportation to and from the conference hotel will be provided, starting at 7 p.m.

Wednesday 5 February

Daily Schedule

8:30 a.m.	**Brief Introduction and Welcome**	**Annegaaike Leopold**

Session 1—Setting the Stage

8:45 a.m.	Science and Policy of Endocrine Disruptors: Sitting at the Crossroads	Glen van der Kraak
9:15 a.m.	2013 – A Critical Year in the Evolution of the Endocrine Disruptor Screening Program: From Vision to Implementation	Mary Manibusan
9:35 a.m.	Endocrine Disruptors – European Chemicals Agency's Role and Activities	Niklas Andersson
9:55 a.m.	EU Industry Perspective on EDC Hazard and Risk Assessment	James Wheeler
10:15 a.m.	**Coffee Break**	

Session 2—Where We Have Been: Data Interpretation and Lessons Learned from Tier 1

10:35 a.m.	Introduction to Session	Jane Staveley
10:40 a.m.	USEPA Endocrine Disruptor Screening Program Tier 1 Battery Overview	Les Touart
11:00 a.m.	Experience at the Organization for Economic Cooperation and Development with Harmonization of Test Methods for the Evaluation of Chemicals for Endocrine Disruption	Anne Gourmelon
11:20 a.m.	**Lunch with Speaker**—Arriving at the Truth: Weight of Evidence for Assessing Risks of Endocrine Disrupting Chemicals in the Environment	Keith Solomon
12:30 p.m.	"Weighing" the Evidence: Relevance and Transparency in the Evaluation of Endocrine Activity	Ellen Mihaich
12:50 p.m.	Weight of Evidence: Evaluating Results from Tier 1 Screening for the USEPA Endocrine Disruptor Screening Program	Amy Blankinship
1:10 p.m.	Cross-Species Conservation of Endocrine Pathways Provides a Basis for Reevaluation of EDSP Tiered Testing Paradigm	Gerald Ankley
1:30 p.m.	Audience Discussion	

Session 3—Where We Are Now: Tier 2 Testing

1:50 p.m.	Introduction to Session	Matt Kern
2:00 p.m.	USEPA's Overview and Status of Endocrine Disruptor Screening Program Tier 2 Tests	Les Touart
2:20 p.m.	The Proposed Tier 2 Medaka Extended One-Generation Reproduction Test (MEOGRT)	Kevin Flynn
2:35 p.m.	Development of the Larval Amphibian Growth and Development Assay (LAGDA)	Sig Degitz
2:50 p.m.	Validation of the Mysid Two-Generation Toxicity Test for the Regulatory Testing of Endocrine Active Compounds	Tim Verslycke
3:05 p.m.	Tier 2 Japanese Quail (*Coturnix japonica*) Avian Toxicity Test	Mary Ann Ottinger
3:20 p.m.	**Coffee Break**	
3:40 p.m.	OECD Perspective on Level 4, Level 5 (Higher–Tier) Studies	Anne Gourmelon
4:00 p.m.	Tier 2 EDSP Assays Viewed Through the Lens of Ecological Risk Assessment	Allen Olmstead
4:15 p.m.	Contract Laboratory Perspective on Higher–Tier Endocrine Testing	Hank Krueger
4:30 p.m.	Panel and Audience Discussion with Session 3 Speakers and Ed Perkins	Matt Kern

5:30–6:30 p.m.	**Poster Social**
7:30–9:30 p.m.	**Conference Banquet**

Thursday 6 February

Daily Schedule

Session 4—Where Are We With Hazard and Risk Assessment?

8:30 a.m.	Introduction to the Session: Perspectives on Hazard and Risk-based Approaches to the Evaluation of Endocrine Disrupting Chemicals	Peter Matthiessen
8:50 a.m.	Regulatory Approaches to Evaluation of Endocrine Disrupting Compounds at Various Government Agencies	Holly Zahner and Jane Staveley
9:10 a.m.	Octylphenol and Nonylphenol as Case Studies for Determining the Relevance of the Endocrine Mode of Action in Human Health and Environmental Assessments	Katie Coady
9:25 a.m.	Magnifying Perceived Risk: A Case Study of Hazard and Risk Assessment of a Pharmaceutical Compound, 17α–Ethinylestradiol (EE2)	Dan Caldwell
9:40 a.m.	Tier 1 Endocrine Disruptor Screening Program (EDSP) Assays and Regulatory Safety Studies Provide a Weight of Evidence that Glyphosate is not an Endocrine Disruptor	Steve Levine
10:00 a.m.	**Coffee Break**	
10:15 a.m.	Non-monotonic dose response curves (NMDRCs) are common after Estrogen or Androgen signalling pathway disruption. Fact or Falderal?	Earl Gray
10:35 a.m.	Modernizing Problem Formulation for Risk Assessment: Potency and Mass Action Govern Endocrine Activity	Chris Borgert
10:50 a.m.	Questions and Comments	
11:05 a.m.	Discussion Leading to a Formal Meeting Statement on the use of Hazard and Risk when Evaluating EDCs	Peter Matthiessen

Session 5—Where Do We Go From Here: Challenges and the Future of EDC Testing

11:35 a.m.	Introduction to the Session	Gerald LeBlanc and Vickie Wilson
11:45 a.m.	Emerging Targets of Endocrine Disruption: The Xenocrine System	Gerald LeBlanc
12:05 p.m.	Emerging Technologies to Assess Endocrine Disruption: Overview of New Assays and Approaches that Show Promise in Evaluating the Endocrine Activity of Chemicals	Seth Kullman
12:25 p.m.	**Lunch (attendees are on their own)**	
1:15 p.m.	Using High-Throughput Methods to Conduct Risk-based Prioritization of Chemicals for the EDSP	Kevin Crofton
1:35 p.m.	Developing In Silico Approaches to Chemical Prioritization for ED Testing within an AOP Context	Patricia Schmieder
1:55 p.m.	In Vitro and In Vivo Testing Methods of Epigenomic Endpoints for Evaluating Endocrine Disruptors	Miriam Jacobs
2:15 p.m.	**Coffee Break**	
2:40 p.m.	Audience Discussion: Questions and Comments	

Meeting Summary and Final Discussion

2:55 p.m.	Meeting summary emerging from the discussion will be developed by Peter Matthiessen and Gary Ankley. The discussion will be facilitated by Jim Lamb.
4:00 p.m.	Meeting Adjourned

About the Speakers

Niklas Andersson

Niklas Andersson is a Toxicologist with the European Chemicals Agency (ECHA) in Helsinki, Finland. He received his MSc in Chemistry from Linköping University, Sweden and PhD in Pharmacology from Lund University, Sweden. Before joining ECHA, he worked for 15 years in academia and business conducting research in the field of sex steroids and energy metabolism. Andersson declares no conflicts of interest.

Amy Blankinship

Amy Blankinship is a scientist with the Environmental Fate and Effects Division with the Office of Pesticide Programs at the USEPA. She has been with the Agency for five years and her responsibilities include evaluating potential ecological risks from the use of pesticides in the environment. Prior to working at the USEPA, she worked as an aquatic biologist at an environmental toxicology laboratory.

Christopher J. Borgert

Christopher J. Borgert is President of Applied Pharmacology And Toxicology, Inc. (APT), a consulting firm that specializes in applied research in the areas of causation analysis, safety assessment and study design. He also holds a courtesy faculty appointment in the Department of Physiological Sciences, University of Florida, College of Veterinary Medicine. He received a bachelor of arts from Kenyon College, Gambier, Ohio, a doctorate in Pharmacology and Therapeutics from the University of Florida, College of Medicine, and completed a postdoctoral fellowship in toxicology at the University of Florida, Center for Environmental and Human Toxicology. He has served on the USEPA Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) as the general representative for small business stakeholders, has testified before Congress regarding the Endocrine Disruptor Screening Program, and has served on numerous national and international expert and peer-review panels including the Society of Toxicology Expert Panel on Chemical Mixtures, OECD Peer-Review Panel for uterotrophic assay validation and ICCVAM Peer-Review Panel for the BG1-Luc estrogen transcription activation assay. He is a past president of the International Society of Regulatory Toxicology and Pharmacology (ISRTP), 2007-2008. His recent publications address weight-of-evidence methods for evaluating endocrine activity and cumulative risk assessments for human exposure to drugs and chemicals.

Daniel J. Caldwell

Daniel J. Caldwell is a Toxicology Fellow in the Environment, Health, Safety and Sustainability Department of Johnson & Johnson and an adjunct associate professor of environmental engineering at Temple University. Caldwell was the chair of the pharmaceutical industry Pharmaceuticals in the Environment (PIE) task force, is a member of the EFPIA PIE Strategy task force, was a founding member of the SETAC Pharmaceutical Advisory Group and served on the steering committee since its inception. He has over thirty years experience in the fields of environmental science, occupational health and toxicology, and he is certified by the American Board of Toxicology (DABT) and the American Board of Industrial Hygiene (CIH).

Kevin M. Crofton

Kevin M. Crofton is the Acting Deputy Director of the National Center for Computational Toxicology of the USEPA in Research Triangle Park, N.C. Crofton received his BA and MS in Zoology at Miami University, Oxford, Ohio, and PhD in Toxicology from the University of North Carolina, Chapel Hill, N.C. He has been a toxicologist at USEPA since 1986 and is an Adjunct Assistant Professor in the Department of Environmental and Molecular Toxicology at North Carolina State University, and in the Curriculum in Toxicology, University of North Carolina at Chapel Hill. His interests include adverse outcome pathways and development of alternative testing methods for endocrine disruption. His current research efforts include development of in vitro and alternative methods for detecting thyroid disrupting chemicals. Crofton's professional activities include membership in numerous scientific societies and participation on many professional review boards. He has presented invited lectures for a variety of government agencies in Europe, Canada and the U.S., and for numerous professional societies and universities. In addition, he has authored or co-authored over 150 peer-reviewed publications.

Sigmund Degitz

Sigmund "Sig" Degitz is a Toxicologist with the USEPA/ORD lab in Duluth, Minn. He received his BS in Biology from Northland College and Ph.D. in Toxicology from the University of Illinois. He has worked at the Duluth lab for nearly 16 years in the area of ectoxicology, most of his career with the agency has been spent studying endocrine disruption in amphibians. Degitz co-led the agencies effort in development of the Tier 1 Amphibian Metamorphosis Assay (AMA). More recently, he has led the development of the Tier 2 Larval Amphibian Growth and Development Assay (LAGDA). He currently serves on the on the Organization for Economic Cooperation and Development, amphibian expert group.

Kevin Flynn

Kevin Flynn is a biologist with the USEPA/ORD lab in Duluth, Minn. He received a bachelor's degree from Augsburg College in Minneapolis, Minn., and a MS from the University of Minnesota-Duluth. He has worked at the Duluth lab for almost 15 years in several areas including the development of protocols for EDC testing in fish, cancer assessments of disinfection byproducts, tissue and cell culture methods to study quinone alkylation and redox cycling, the development of molecular tools in EDC testing and confocal/multi-photon microscopy.

Anne Gourmelon

Anne Gourmelon has been head of the OECD Test Guidelines Programme in Paris since 2013. For more than 10 years, Gourmelon has been involved in the international validation and harmonization of test methods and in the development of guidance material for the detection of potential endocrine disrupters in wildlife species at the OECD. Gourmelon has also worked on the OECD Cooperative Chemicals Assessment Programme for the evaluation of high production volume chemicals. She is an environmental scientist by education and has studied in France and obtained a master's degree in environmental toxicology from the University of Wageningen in the Netherlands.

L. Earl Gray Jr.

Earl Gray is a senior scientist in the Reproductive Toxicology Branch in the TAD, NHEERL, ORD, USEPA research laboratory in Research Triangle Park, N.C. He has worked at the USEPA since 1976. The research is focused on how exposure to individual pesticides and toxic substances and mixtures of chemicals alters reproductive development in mammalian and other vertebrate reproductive systems.

Miriam Jacobs

Miriam Jacobs is a senior scientist at Public Health England, with responsibilities in the chemical regulatory field, particulary with respect to the OECD Test Guideline Programme. Specialities include endocrine disruption, reproductive toxicology and alternatives to animal testing. She has also worked for the European Food Safety Authority, the European Commission Joint Research Centre, the OECD and the UNDP. She sits on several Scientific Advisory Boards (European, US, Japan) and is on the expert rosters for the Joint FAO/WHO Expert Committees on Food Additives and Pesticide Residues.

Matt Kern

Matt Kern is an ecotoxicologist with over 16 years of experience in the area chemical registration support. This includes the management of aquatic and terrestrial ecotoxicology GLP laboratories, conducting of environmental risk assessments and general product defense. During this period, he has managed numerous crop protection products through development and registration review phases. Risk assessments conducted have included partnering with exposure and environmental fate experts for both screening level and higher-tiered assessments. He has partnered with contract research organizations to complete a wide range of ecotoxicity studies. This has included standard and specialized studies designed to address specific scientific and regulatory concerns. He has been directly involved in the validation and conduct of studies designed to meet the requirement of the USEPA Endocrine Screening Program (EDSP). He has actively participated in a number of industry and multi-stakeholder working groups and societies (e.g., CropLife America, OECD expert teams, SETAC). He currently supports clients as an ecotoxicology consultant with Waterborne Environmental. He is actively managing numerous ecotoxicology studies and premier CROs and conducting both deterministic and probabilistic risk assessments supporting various sponsors.

Seth W. Kullman

Seth Kullman is an Associate Professor in the Department of Biological Sciences and Program in Environmental and Molecular Toxicology at North Carolina State University. He received his PhD from the University of California at Davis in Pharmacology and Toxicology (1996). He subsequently served as assistant director of the UC Davis Ecotoxicology program. Kullman joined the faculty of the Integrated Toxicology Program at Duke University (2000) and served as director of the Duke University Superfund Center Functional Genomics Core (2004-2007). Kullman has a long-standing history of employing small aquarium fish models in mechanistic toxicity studies with an emphasis on generating a comprehensive understanding of how genetic and environmental factors interact influencing human and environmental health. His research involves elucidating the mechanism by which environmental exposures modulate ligand activated transcription factors (nuclear receptors and AhR) associated with embryogenesis, fetal origins of adult disease and endocrine disruption. Kullman has over 50 peer-reviewed publications and book chapters. He has been a visiting scholar at the Mount Desert Island Biological Laboratory. He has participated in proposal review panels for the NSF, NOAA and the National Institute of Environmental Health Sciences. He has participated as an ad hoc member for scientific review with the USEPA, FIFRA-Scientific Advisory Panel Endocrine Disruptor Screening Committee, Water Environment Research Foundation Project Advisory Committee, American Water Works Association Research Foundation Project Advisory Committee, and he is a full member of SETAC.

James C. Lamb

Jim Lamb has more than 30 years of experience specializing in toxicology, risk assessment and regulatory policy. After getting his PhD at the University of North Carolina, Lamb has worked on hormonally active agents in the environment ("endocrine disruptors") since 1976 when he joined the National Institute of Environmental Health Sciences. Lamb moved to the National Toxicology Program, then to USEPA as the Special Assistant for Pesticides in the Office of Pesticides and Toxic Substances. He has been a consultant in this area for more than 25 years and is currently the Center Director of Exponent's Toxicology and Mechanistic Biology Center. Lamb has served on numerous scientific panels for government and private organizations. A few of the organizations that he has served include several National Academy of Sciences Committees, the U.S. Delegation to the Organization for Economic Cooperation and Development (OECD) and the International Life Sciences Institute (ILSI). He is now Past-President of the Academy of Toxicological Sciences. He was honored by the SOT's Mid-Atlantic Society of Toxicology as their 2011 Ambassador of Toxicology.

Annegaaike Leopold

Annegaaike Leopold is an ecotoxicologist and has worked in the contract research field for 24 years. She began her career in aquatic toxicology managing and directing laboratory studies and subsequently specialised in avian toxicology working in the laboratory for five years and as a consultant subsequent to that. She has managed and coordinated ecotoxicology programmes for Wildlife International's European clientele for 14 years and is now in the role of Director of Global Business Development for Wildlife International, PTRL-Europe and PTRL-West, all part of EAG, Inc. She has been deeply involved in OECD guideline development in the fields of avian and endocrine toxicology. She has worked on the development of guidance documents for avian and endocrine risk assessment for the European Food Safety Authorities. Leopold chaired the successful SETAC Europe Scientific Symposium on Endocrine Disruption Testing and Evaluation held in Brussels, Belgium, in October 2012.

Peter Matthiessen

Peter Matthiessen is an independent consulting ecotoxicologist, who has been conducting research and development in the field of endocrine disruption since the mid-1980s. He has particular expertise in studying the effects of estrogens and their mimics on fish, and the effects of organotins on molluscs. He is currently involved in research revealing that treated sewage discharges are able to damage the hormonal response to stress in fish. Matthiessen is a member of the UK Hazardous Substances Advisory Committee (HSAC) and co-chair of the OECD Validation Management Group for Ecotoxicity Tests (VMG-eco). He has been closely involved in the development and international standardization of assays for the detection and hazard assessment of endocrine disrupters and has helped develop detailed guidance for how the results of these assays should be interpreted using weight-of-evidence procedures. He is keen to develop further guidance which can help chemical companies and regulatory authorities to conduct environmentally sound hazard and risk assessments of these substances.

Ellen Mihaich

Ellen Mihaich has worked in the pesticide/chemical industry for more than 23 years. She is the owner and president of Environmental and Regulatory Resources, LLC, an environmental consulting company in Durham, N.C. Prior to this position, she worked for Rhone-Poulenc and at Rhodia as an environmental toxicologist responsible for pesticide/chemical development, testing and risk assessment. Mihaich has been involved in test guideline development and endocrine-related activities for many years. Among the many activities in this area, she is a Business and Industry Advisory Committee representative to the OECD Ecological Validation Management Group for endocrine testing. She has also been an invited participant on three Intergovernmental Coordinating Committee on Validation of Alternative Methods panels on in vitro testing methods for endocrine active compounds and the use of the Frog Embryo Teratogenesis Assay Xenopus assay in human health assessment. She is the scientific coordinator for the Endocrine Policy Forum, a consortium of List 1 Test Order recipients and interested stakeholders. Mihaich received a BA from Wellesley College and both MS and PhD degrees in environmental toxicology from Duke University, where she currently holds an adjunct appointment and teaches a graduate-level course in risk assessment. She is a past president of SETAC. Mihaich is also a Diplomate of the American Board of Toxicology.

Allen W. Olmstead

Allen Olmstead received his PhD in Toxicology from North Carolina State University in 2003, where he studied the sexual differentiation of *Daphnia magna* and it's perturbation by xenobiotics via methyl farnesoate signaling. This was followed by a postdoc modeling the effects of chemical mixtures. From 2006 to 2012, Olmstead worked at the USEPA in Duluth, Minn., developing protocols for long-term testing of endocrine-active compounds on amphibians with a focus on the hypothalamus–pituitary–gonadal axis. This work led to the development of the Larval Amphibian Growth and Development Assay, which has been put forth as a US Endocrine Disruption Screening Program Tier II assay. During this time, Olmstead also worked on fish population models for use in risk assessment. Since 2012, Olmstead has worked for Bayer CropScience in the Environmental Toxicology and Risk Assessment group, where he focuses on ecotoxicology and risk assessment of bees. He is currently a member of the OECD Test Guideline Program Amphibian expert group.

Mary Ann Ottinger

Marry Ann Ottinger is a Professor of Reproductive Physiology and Neuroendocrinology at the University of Maryland, where she also earned her PhD. She is currently Associate Vice Chancellor and Associate Vice President for Research at the University of Houston. Her professional experience includes a postdoctoral fellowship at the National Institute of Health in Bethesda, MD. During a sabbatical, Ottinger spent time in the Department of Physiology at the University of Maryland School of Medicine and held a position at the Laboratory of Experimental Gerontology in the Nathan Shock Gerontology Research Center, National Institute on Aging, National Institute of Health, in Baltimore, Md. Ottinger was also a Research Associate at the National Zoo, Smithsonian Institution. Ottinger's administrative accomplishments begin with being Associate Program Director of the Psychobiology Program at the National Science Foundation. She was also a Coordinator of collaborative reproductive studies between the Laboratory of Experimental Gerontology, National Institute on Aging, National Institute of Health and Oregon National Primate Research Center. Her research interests include the study of the neuroendocrine mechanisms that direct reproductive endocrine and behavioral responses in development and aging. Studies include wildlife and domestic birds, with a focus on enhancing and extending reproductive lifespan for conservation applications. Ongoing studies consider the effects of nutrition on reproductive and metabolic system aging in the rhesus macaque. The role of aging and effects of estrogens in neurodegenerative disease is also under study in a mouse model of Alzheimer's disease. These interests are co-mingled in studies on the consequences of early embryonic and lifetime exposures to environmental endocrine disruptors on reproductive aging and overall individual health in wildlife and domestic species. Significant accomplishments include her time as co-editor of Avian Biology Reviews. She has served on a number of boards and committees including being a member of the Board of Directors and President at the Federation of Animal Science Societies. Ottinger served on the OECD Avian Expert Toxicology Testing Work Group and Advisory Committee and was on the Peer Review Panel and Public Hearing for the USEPA Ecological Risk Assessment for Housatonic River Area. Ottinger has more than 250 published peer reviewed publications and published abstracts.

Henry Krueger

Henry "Hank" Krueger is the Director of Aquatic, Plant and Insect Toxicology at Wildlife International, Ltd. in Easton, Md. He has served in this position for the last 16 years and manages business and financial affairs, as well as provides technical direction in program coordination and development. His background is in both aquatic and terrestrial ecotoxicology and early in his career at Wildlife International served as Director of Field Programs, where he had overall responsibilities for directing activities of mesocosms, terrestrial and aquatic field studies, as well as plant studies conducted in greenhouses. He obtained his MS from Central Michigan University and his PhD from the University of Wyoming, where he worked at the US Fish and Wildlife Cooperative Research Unit. He has been involved with endocrine studies for the past 15 years and has served as project manager for endocrine studies that have included mysid, frog and fish.

Mary Manibusan

Mary Manibusan has been with the USEPA for 15 years. She is the current Director of the Endocrine Disruptor Screening Program in the Office of Science, Coordination and Policy. Manibusan served as Chief of the Risk Management and Implementation Branch in the Pesticide Re-evaluation Division and Chief of the Toxicology and Epidemiology Branch (TEB) in the Health Effects Division of the Office of Pesticide Programs at USEPA. She first joined USEPA's Office of Water in the Office of Science and Technology's Health and Ecological Criteria Division as a human health toxicologist on complex risk assessments including disinfection byproducts, perchlorate and methylmercury. Manibusan was also Senior Toxicologist with the Office of Research and Development, National Center for Environmental Assessment (IRIS program), where she focused on prioritization of IRIS chemicals assessments and the development of modes-of-action and toxicity pathways and later served as the ORD Health Coordinator during the emergency response efforts for Hurricanes Katrina and Rita.

Ed Perkins

Edward J. Perkins received a BS in 1983 in Genetics from the University of Illinois in Champaign-Urbana, Ill. He received his PhD in 1987 in Genetics and Cell Biology from Washington State University in Pullman, Wash., studying biodegradation of the herbicide 2,4-D by bacteria. He is currently Senior Scientist in Environmental Networks and Genetic Toxicology at the US Army Engineer Research and Development Center (ERDC). His research activities include understanding the biological effects of military-relevant materials (e.g., explosives, nanomaterials, metals), toxicity of industrial chemicals to animals, biological networks, bioinspired material design and tracking invasive species with environmental DNA. He leads an active and diverse group of scientists in basic and applied research efforts in these areas. One of his current responsibilities is leading a multi-disciplinary ERDC research cluster focusing on the understanding and modeling the mechanisms by which military materials cause toxicity. Perkins provides guidance and direction to ERDC's Army programs in environmental quality. He serves as the ERDC and Army lead for biological networks, where he develops and oversees ERDC efforts in network science and how these translate to engineered systems. He serves as mentor to junior scientists in ERDC to assure development of world-class science. In addition to his research on military materials, he advises the Organization for Economic Cooperation and Development on harmonization of chemical regulatory approaches and the USEPA on screening chemicals for toxicity. Perkins has authored or co-authored more than 100 peer-reviewed journal publications and 4 book chapters. He is an active member of several national and international organizations including SETAC, International Society for Computational Biology, the Society of Toxicology (SOT) and is a Past-President of the Mid-South Bioinformatics and Computational Biology Society.

Patricia Schmieder

Patricia "Pat" Schmieder is a Toxicologist with the USEPA Office of Research and Development lab in Duluth, Minn. She received her BS from the University of Wisconsin-Stevens Point, her MS in Environmental Science from Rutgers University and her PhD in Toxicology from Oregon State University. She has worked at the Duluth USEPA lab for more than 25 years in areas including fish toxicokinetics, lethal body burdens, fish acute toxicity syndromes, in vitro assays for reactive chemical toxicities, adverse outcome pathways, estrogenicity assays and QSAR predictive systems, metabolism research, metabolism pathways knowledgebase and data analysis tools. Schmieder has been engaged in the development of tools for decision support for chemical risk assessments both nationally and internationally including the MetaPath metabolism pathway analysis tool and the USEPA's Estrogen Receptor Expert System for chemical prioritization.

Jane Staveley

Jane Staveley is a Senior Managing Scientist with Exponent, Inc. She has 35 years of experience as a consultant in environmental toxicology, ecological risk assessment and product stewardship, working with clients in both the public and private sector on a broad variety of environmental issues. She has extensive experience in ecotoxicology testing and risk assessment for industrial chemicals, pesticides, pharmaceuticals and consumer products. Staveley has prepared a number of environmental assessments for veterinary and human pharmaceuticals to meet regulatory requirements in the U.S. and Europe. She has monitored ecotoxicology testing programs and conducted ecological risk assessments pursuant to pesticide registration under the Federal Insecticide, Fungicide and Rodenticide Act. She has also evaluated ecotoxicology tests and prepared weight-of-evidence arguments submitted under the USEPA's Endocrine Disruptor Screening Program. Staveley obtained a BS in Biology at the College of William and Mary and a MSPH in Environmental Chemistry and Biology at the University of North Carolina. She has been active in SETAC throughout her career, serving on the SETAC World Council from 2006–2011 and as President in 2010. She has authored or co-authored more than 50 publications and presentations.

Les Touart

Les Touart is a senior ecotoxicologist with the USEPA Office of Chemical Safety and Pollution Prevention in Washington, D.C. He received his BS in marine biology and MS in biology from the University of West Florida and PhD in environmental biology from George Mason University. He has served with NOAA National Marine Fisheries Service and has 36 years with USEPA, initially serving at the USEPA Gulf Breeze laboratory performing marine organism toxicity tests and then several years in the Office of Pesticide Programs performing ecological risk assessments and developing test guidelines from molecular-based in vitro assays to large community-based aquatic mesocosms. Touart has served for the past 18 years in support of the Endocrine Disruptor Screening Program and has overseen the validation efforts for all the ecological test methods. He has been very active internationally with the Organization for Economic Cooperation and Development and serves on several expert groups in support of ecological test guidelines development.

Glen Van Der Kraak

Glen Van Der Kraak is a Professor in the Department of Integrative Biology and the Associate Dean for Research in the College of Biological Science at the University of Guelph. He has published more than 280 referred journal articles, reviews and book chapters and has written or edited four books. He has extensive experience in the testing of chemicals and complex effluents for effects on the reproductive physiology of fish and amphibians. Van Der Kraak won the Award for Excellence in Research through the University of Guelph chapter of Sigma Xi in 2002. He has served on two panels of the World Health Organization's International Program on Chemical Safety, evaluating risks posed by endocrine disrupting chemicals and methods of integrating human health and ecological risk assessments. He was a member of the Royal Society of Canada Expert Panel on Environmental and Health Impacts of Canada's Oil Sands Industry. He has frequently served as an advisor to the USEPA including serving as a member of the Endocrine Disruptors Methods Validation Sub Committee, the Endocrine Disruptors Methods Validation Advisory Committee and the Board of Scientific Counsellors that reviewed the USEPA's Endocrine Disruptors Research Program.

Tim Verslycke

Tim Verslycke has 15 years of combined consulting and academic research experience in ecological risk assessment. He has authored more than 30 peer-reviewed journal articles, and he speaks regularly at scientific conferences on this and other topics. His areas of expertise include evaluating environmental risks at contaminated sites, conducting environmental safety assessments of endocrine disruptors, pharmaceuticals and personal care products, pesticides and other industrial chemicals, and regulatory ecotoxicity testing. Verslycke also holds a research position in the Biology Department at the Woods Hole Oceanographic Institution in Mass. He is currently a member of the SETAC Pharmaceutical Advisory Group Steering Committee and President of the SETAC North Atlantic Chapter. He is also a former scientific advisor to the Center for Health and Environment of the Flanders Regional Government in Belgium and a former member of the scientific committee of the Flanders Marine Institute in Belgium.

James R. Wheeler

James Wheeler works as an ecotoxicologist for Product Safety at Syngenta's Jealott's Hill International Research Centre, UK. His work focuses on aquatic ecotoxicology testing and specifically fish endocrine test methods. Wheeler is a member of the OECD's Validation Management Group for ecotoxicity tests and fish drafting group. He is also a member of a number of industry groups working in the area of endocrine disruption. Before joining Syngenta, he completed his PhD on endocrine disruption in fish at the University of London.

Vickie S. Wilson

Vickie Wilson is a research biologist with the USEPA Office of Research and Development lab in Research Triangle Park, N.C. Currently she serves as Chief of the Reproductive Toxicology Branch within the Toxicity Assessment Division. Wilson received her BS in Food Chemistry from Framingham State College, Mass., and her PhD in Toxicology from the Department of Molecular and Environmental Toxicology at North Carolina State University. She has worked at the USEPA lab for 13 years. During that time, her research has focused on several areas including development of in vitro assays for defining mechanism of action of environmental compounds, the application of those assays as screening tools for hormonal activity in environmental samples, and the assessment of endocrine disrupting chemicals and their impact on the cellular and molecular mechanisms of toxicant induced abnormal reproductive development using in vitro, ex vivo and in vivo models. Her work on these and related topics has resulted in more than 70 publications.

Holly Zahner

Holly Zahner is a Toxicologist with the US Food and Drug Administration's Center for Veterinary Medicine (FDA/CVM). She received her BA in Biology from St. Mary's College of Maryland, and an MS and PhD in Environmental Toxicology from the Department of Biological Sciences at Clemson University. Zahner has worked at the FDA/CVM for more than four years evaluating the environmental safety of a variety of new animal drugs applications, generic abbreviated new animal drug applications, and import tolerances for food-producing and companion animals. She received the FDA Outstanding Service Award in 2011 for her work on developing center-level policy and risk assessment approaches to evaluate the potential environmental risks of animal drugs containing steroid hormones. Zahner became Leader of the Environmental Safety Team at FDA/CVM in May of 2013.

Abstracts

Endocrine Disruption: Chemical Testing and Risk Assessment Approaches and Implications

The following pages comprise the abstracts for the poster presentations of the SETAC North America Focused Topic Meeting, Endocrine Disruption: Chemical Testing and Risk Assessment Approaches and Implications, conducted at the US Environmental Protection Agency Campus in Research Triangle Park, North Carolina, 4–6 February 2014. The abstracts are reproduced as accepted by the Steering Committee.

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Glen Van Der Kraak | *University of Guelph*

Science and Policy of Endocrine Disruptors: Sitting at the Crossroads

In 1991, scientists meeting at Wingspread issued the statement that "Many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wildlife, and humans". This served as one of the origins of the endocrine disruptor hypothesis and the literally thousands of publications that followed. To date there remains considerable uncertainty and controversy as to how to use this information in regulation that is protective of apical endpoints including growth, reproduction and development. In 2002 the World Health Organization issued a report on the State of the Science of Endocrine Disrupting Chemicals which included an objective and transparent framework for assessing the relationship between potential endocrine disruptors and health outcomes. In a follow-up report published in 2012, the World Health Organization in collaboration with the United Nations Environment Programme moved away from a weight-of-evidence approach for the evaluation of data on endocrine disruption and used "best professional judgment" and often did so without evaluating the totality of the evidence. Over the last 20 years, much progress has been made in the development of standardized and harmonized test guidelines for regulatory application that address the effects of chemicals on estrogen, androgen, and thyroid dependent pathways in lab animal and selected wildlife species. These studies are time consuming and costly from both a monetary and an animal use perspective and still come with their own technical challenges including uncertainty as to the predicitivity of test results and coverage of only a subset of endocrine effects. On the basic science side there has been much progress made in defining the mode of action of chemicals on aspects of endocrine physiology, but there continues to be gaps in making the linkages between effects seen at the molecular and cellular levels of biological organization and apical endpoints. This later point is becoming highly charged given the pending legislation in Europe that would see regulation of agents that show endocrine-mediated effects in some experimental systems including in silica, in vitro and in vivo models. So while there have been major strides in identifying and defining the actions of endocrine disrupting chemicals, the field struggles on how to best use this information in regulatory decision-making.

Mary Manibusan | *USEPA*

2013 – A Critical Year in the Evolution of the Endocrine Disruptor Screening Program: From Vision to Implementation

In 2013, the Endocrine Disruptor Screening Program underwent a series of four highly significant external FIFRA Scientific Advisory Panel (FIFRA SAP) peer reviews on the critical science supporting the program: 1) the application of advanced computational toxicological methods for chemical prioritization; 2) the performance of the Tier 1 screening assays and battery for the initial list of chemicals; 3) scientific validation of the Tier 2 ecological species test methods, inclusive of bird, frog, fish and invertebrate; and 4) interpreting Tier 1 and other scientifically relevant data in accordance with the EDSP Weight of Evidence Guidance, issued in September of 2011. Following these four external peer reviews, the agency will be carefully considering all of the FIFRA SAP recommendations for the EDSP and other technical modifications to the program as we advance forward. Based on culmination of test method validation efforts and data reviews in 2013, the agency will, over the next few years, be focusing on programmatic implementation; this will proceed with the scientifically rigorous technical review of all Tier 1 assay results from the initial list of pesticidal chemicals that received EDSP Tier 1 orders in 2009, and review of that collective screening data, along with other scientifically relevant data to complete the regulatory weight of evidence decisions. If the weight of scientific evidence indicates a need for Tier 2 longer term testing, the agency will issue test orders accordingly. And to the extent that the Tier 2 data indicate a risk of concern, the agency can/will act accordingly to address that risk scenario. While the agency has invested resources in developing and validating screening and definitive test methods, the need for a more comprehensive review of state-of-the-science and emerging technologies for toxicity testing has been recognized. In this recognition, the USEPA had issued its plan for how the agency will incorporate computational toxicological tools into the Endocrine Screening Program and this is described more specifically within the Endocrine Disruptors Screening Program for the 21st Century: (EDSP21 Work Plan), issued on 30 September 2011.

Niklas Andersson | *European Chemicals Agency*

Endocrine Disruptors – ECHA's Role and Activities

The European Chemicals Agency (ECHA) is managing the implementation of the REACH (1), CLP (2) and Biocidal Products (3) Regulations to ensure consistency at the EU/EEA level as well as credible science-based decision-making. Endocrine disruptors (ED) are addressed in the REACH Regulation in the context of restriction and authorisation. Under the Biocidal products Regulation EDs are addressed in the context of the approval of active substances and exclusion criteria. Under REACH, substances having endocrine disrupting properties may be identified, on a case-by-case basis, as substances of very high concern (SVHC) and added to the REACH Candidate List. Authorisation requirements apply to SVHCs that are included in Annex XIV of REACH. Currently four EDs have been placed on the Candidate list of SVHCs. In order to facilitate identification of EDs for regulatory action, efforts are ongoing at EU level to develop a common set of criteria for identification of EDs under the REACH, Biocidal Products and Plant Protection Products Regulations. The standard information requirements under REACH cover some of the tests from the OECD conceptual framework for the assessment of EDs, but they are not comprehensive and do not generally include mechanistic data. ECHA may in the process of dossier evaluation only request standard information, while the Member State Competent Authorities conducting substance evaluation may also request additional information that goes beyond the mainly tonnage-dependent standard information requirements of REACH. Therefore, substance evaluation under REACH is best suited to investigate ED properties. The CLP Regulation does not address ED directly. However, the provisional criteria under the Plant Protection Products and Biocidal Products Regulations as well as the on-going discussions to develop criteria for identification of EDs for regulatory action make reference to the CLP criteria for classification and labelling. Therefore, CLP may play an indirect role in the identification of EDs. Further Guidance and information on all processed co-ordinated by ECHA are available on the Agency's website:

http://echa.europa.eu. Disclaimer: This text reflects the view of the author and does not necessarily represent the official opinion of the European Chemicals Agency (ECHA). 1. Regulation (EC) No 1907/2006 on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH); 2. Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures; 3. Regulation (EU) No 528/2012 on making available on the market and use of biocidal products.

James R. Wheeler | *Syngenta Ltd*

EU Industry Perspective on Endocrine Disruption Hazard and Risk Assessment

In recent years, endocrine disruption has become a topic of increasing public and regulatory concern. This presentation will outline some of the current approaches being taken by European regulatory agencies and industry working groups in order to define suitable criteria for the identification and management of endocrine disrupting chemicals. Plant protection products are likely to be impacted by recent changes which will create hazard based cut-off (ineligibility for registration) criteria for endocrine disrupting properties and so are used here to illustrate the approaches under development. The background to the changes in EU legislation dealing with endocrine disruptors will be discussed. For Plant Protections Products the cut-off criterion poses serious practical implications. It is currently, poorly defined and open to varying interpretations that may lead to inappropriate classification and removal from the market of useful substances. Highlighting the need for robust and scientifically defensible criteria for the identification of endocrine disrupting properties. Impact assessments of the potential effect of the criteria on the EU plant protection industry will also be discussed.

Les Touart | *USEPA*

USEPA's Endocrine Disruptor Screening Program Tier 1 Battery Overview

USEPA completed validation of a battery of 11 assays and began issuing test orders on an initial list of prioritized chemicals in 2009. A brief review of the history and rationale behind the assays which make up the battery is presented. DISCLAIMER: The opinions presented are those of the author and may not reflect EPA policies.

Anne Gourmelon | *Organisation for Economic Cooperation and Development (OECD)*

Experience at the OECD with Harmonization of Test Methods for the Evaluation of Chemicals for Endocrine Disruption

The protection of human health and the environment from endocrine disrupters (ED) is currently a high priority for regulatory authorities in several OECD countries/regions. Indeed, the OECD Test Guidelines Programme has spent approximately half of its resources since 1996 to develop test guidelines and other tools to support countries' needs related to testing and assessment of chemicals for endocrine disruption. These works have been made possible because some OECD countries like the United States had a dedicated programme, a clear goal and resources to undertake the validation studies. OECD Test Guidelines are standardised, validated and harmonized test methods used across countries adhering to the Mutual Acceptance of Data, a government-to-government agreement aiming at reducing non-tariff barriers to trade and avoiding duplicative testing. After more than 15 years working on the validation and development of methods for screening and testing chemicals for endocrine disruption, a Conceptual Framework functioning as a toolbox has been developed and refined; more than 10 OECD Test Guidelines specific to ED have been validated and adopted; a large number of guidance and reviews documents, validation reports and workshop report have been agreed and published in support of the Test Guidelines. A few long-term test methods for wildlife species are still under discussion and the OECD is keen on developing harmonized methods for these important and resource-intensive assays. In looking towards the future, OECD countries are conscious of the need to assess more chemicals more efficiently. OECD is providing a forum to discuss and harmonize ways to integrate new technologies and novel approaches in the testing and assessment of ED, based on knowledge of the modes of action leading to adverse outcomes.

Keith R. Solomon | *University of Guelph*

Arriving at the Truth: Weight of Evidence for Assessing Risks of Endocrine Disrupting Chemicals in the Environment

Additional Authors: G. van der Kraak, Department of Integrative Biology, University of Guelph; M. Hanson, Department of Environment and Geography, Faculty of Environment, Earth, and Resources, University of Manitoba; W. Kloas, IGB Berlin; A. Hosmer, Syngenta Crop Protection LLC

Information and data on endocrine disrupting chemical from studies published in the open literature is increasing being used for assessment purposes by regulatory agencies in many jurisdictions, including North America and Europe. Because most of these studies are not conducted to the standards required of GLP studies required by regulatory agencies, there is a need to assess these studies for their quality and relevance to the regulatory endpoints being considered. In addition, the data from these studies need to be integrated with those from studies conducted for regulatory purposes. Weight of evidence (WoE) offers a consistent, objective, and transparent method for assessing these studies and including them in risk assessment and assignment of causality. These data also need to be integrated into lines of evidence that inform adverse outcome pathways (AOPs) and lines of evidence related to apical endpoints such as survival, growth, development, reproduction. The WoE process that was developed involves an assessment of the strength of the study. Here, various aspects of the methods, such as the experimental design and conduct, the use of appropriate controls and measures of exposures, the inclusion of environmentally realistic concentrations. Number of concentrations, quality control, and transparency of data are scored with a numerical score. The relevance of the responses was assessed by scoring statistical significance, concentration or dose-response, relevance to an appropriate apical endpoint, and a biologically plausible mechanism. The WoE process was inclusive and no studies, except those with mixtures where the individual components were not tested individually were included. Results were presented graphically for easy interpretation. The need for WoE and an illustration of one method for conducting these analyses will be presented with the herbicide atrazine as case example.

Ellen Mihaich | *Environmental and Regulatory Resources*

"Weighing" the Evidence: Relevance and Transparency in the Evaluation of Endocrine Activity

Additional Authors: C. Borgert, APT Inc.; L. Stuchal, University of Florida; L. Ortego, Bayer CropScience, S. Marty, Dow Chemical Co.; J. Brausch, BASF

There are several key elements to a scientifically based weight of evidence (WoE) process, including the development of a priori specific hypotheses, a systematic review of relevant literature including an evaluation of data quality and reliability, and an assessment of consistency, specificity and reproducibility of effects. These elements all focus on the relevance and transparency of the data and the assessment process. To this end, a conceptual framework for conducting weight of evidence (WoE) evaluations of endocrine screening and testing data (Borgert et al., 2011) was developed. The framework was designed to be broadly applicable, but with an emphasis on data emerging from the USEPA's Endocrine Disruptor Screening Program (EDSP), and specifically tailored to accommodate endpoints measured in the eleven Tier 1 Endocrine Screening Battery assays. The framework calls for a transparent process whereby specific hypotheses are formulated and data quality is evaluated with respect to overarching scientific principles, including primary validity of the measurements, reliability of data reporting, and probative capability of the study design to evaluate causation. The framework also calls for assigning quantitative weighting factors to assay endpoints (WREL) that reflect their relevance for deciding specific hypotheses in relation to in vivo endocrine activity and effects. This is an important step in the WoE framework as it explicitly recognizes the fact that some assays are very specific and sensitive for evaluating certain hypotheses related to endocrine activity but may be irrelevant for evaluating others. For example, an increase in thyroid follicular hyperplasia, as assessed in the amphibian metamorphosis assay, would be expected to have a very high WREL for evaluating the hypothesis that a chemical has potential thyroid antagonist activity, but would have a very low WREL for evaluating the hypothesis that a substance has potential estrogen or androgen agonist or antagonist activity. Case studies of rank ordered WREL values for evaluating Tier 1 EDSP hypotheses will be presented for compounds tested in the validation and/or list 1 phase of the EDSP.

Amy Blankinship | *USEPA*

Weight of Evidence: Evaluating Results from Tier 1 Screening for the USEPA Endocrine Disruptor Screening Program

Additional Authors: J. Rowland, G. Akerman, C. Aubee, T. Steeger, J. Liccione, Office of Pesticide Programs, USEPA

The USEPA issued its weight-of-evidence (WoE) guidance document in 2011 for evaluating the results of Endocrine Disruptor Screening Program (EDSP) Tier 1 battery and other scientifically relevant information (OSRI) to identify whether Tier 2 testing is warranted. The guidance document presents a hypothesis-based approach that begins with an evaluation of each Tier 1 study's quality and relevance in addressing the questions for the chemical of interest, and guidance on how to assemble and integrate multiple lines of evidence (EDSP Tier 1 assays and OSRI), including peer-reviewed open literature studies), for that chemical. Additionally, the application of the WoE process incorporates the conceptual framework of an adverse outcome pathway (AOP) where various levels of biological organization are considered. Thus, Tier 1 screening combines other relevant evidence (e.g., 40 CFR Part 158 guideline studies) using a WoE analysis to determine whether or not a test chemical may require a more comprehensive Tier 2 testing or a more targeted and tailored approach to address specific uncertainties. This approach was presented by the USEPA at a FIFRA Scientific Advisory Panel meeting in July 2013. This presentation will discuss the different aspects of the USEPA 2011 WoE document and how they were applied within the context of an AOP to a case study which is based on data from a chemical in List 1. Disclaimer: The opinions presented are those of the authors' and may not reflect USEPA policies.

Gerald Ankley | *USEPA*

Cross-Species Conservation of Endocrine Pathways Provides a Basis for Reevaluation of EDSP Tiered Testing Paradigm

Additional Authors: L.E. Gray, USEPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Toxicity Assessment Division

Many structural and functional aspects of the vertebrate hypothalamic-pituitary-gonadal (HPG) axis are known to be highly conserved, but the relative significance of this from a regulatory toxicology perspective has received comparatively little attention. High-quality data generated through development and validation of Tier 1 tests for the USEPA Endocrine Disruptor Screening Program (EDSP) offer an unique opportunity to compare responses of mammals versus fish to chemicals that may affect shared pathways within the HPG axis. Our analysis focused on data generated with model chemicals that act (primarily) as estrogen receptor agonists (17α-ethynylestradiol, methoxychlor, bisphenol A), androgen receptor agonists (methyltestosterone, 17β-trenbolone), androgen receptor antagonists (flutamide, vincolozolin, p,p'-DDE) or inhibitors of different steroidogenic enzymes (ketoconazole, fadrozole, fenarimol, prochloraz). All 12 chemicals had been tested in the EDSP fish short-term reproduction assay (FSTRA) and in one or more of the four in vivo Tier 1 screens with rats (Uterotrophic, Hershberger, male and female pubertal assays). In most cases there was high concordance between the fish and rat assays with respect to identifying chemicals that impacted specific HPG pathways of concern, with the test chemicals producing positive results in the fish and one or more of the rat tests. However, some assays were clearly superior to others in terms of detecting specific pathways; for example, the effects of inhibitors of steroid hormone synthesis were most obvious in the FSTRA, whereas the activity of androgen receptor antagonists were clearest in the Hershberger and male pubertal assays. Based on our analysis it may be possible to use just two of the current Tier 1 tests, the FSTRA and the male pubertal assay, to ensure full coverage of HPG axis pathways of concern. Based on this, we propose that these two tests could serve as initial "gate keeper" assays, following which unknown chemicals may be exempted from further testing (negatives) or (when positive) subjected to additional, confirmatory analyses with other existing Tier 1 assays. This would greatly enhance throughput of chemicals through initial testing, both in terms of resource utilization and timing. This abstract does not represent the views of the USEPA.

Les Touart | *USEPA*

USEPA's Endocrine Disruptor Screening Program Tier 2 Ecotoxicity Test Methods

USEPA established the Endocrine Disruptor Screening Program (EDSP) in response to a US Congressional mandate "to determine whether certain substances may have an effect in humans that is

similar to an effect produced by a naturally occurring estrogen, or such other effects as [USEPA] may designate" [21 U.S.C. 346a(p)]. As part of the EDSP, USEPA is validating assays to identify and characterize the endocrine activity of pesticides, commercial chemicals, and environmental contaminants, specifically in relation to estrogen, androgen, and thyroid hormones. This talk will present a brief historical summary of the development and validation of the candidate test methods including a mammalian two-generation test, a Japanese quail two-generation test, the Larval Amphibian Growth and Development Assay (LAGDA), a medaka multi-generation test, and an invertebrate test. Although a medaka multi-generation test was the principal fish method considered, an abbreviated medaka reproduction test was also proposed. Additionally, a mysid two-generation toxicity test is recommended as the preferred invertebrate in vivo Tier 2 EDSP test, but a harpacticoid copepod reproduction and development test was also considered as a potential alternative or option. The reasoning and judgments leading to the various studies that were conducted as part of the development, demonstration, and validation of the various test methods. In addition, the outcome and recommendations of a FIFRA SAP review (www.epa.gov/scipoly/sap/meetings/2013/june/062513minutes.pdf) of the proposed methods and public comments of the revised methods will be summarized and discussed. Status of the final test guidelines will be presented. DISCLAIMER: The opinions presented are those of the author and may not reflect EPA policies.

Kevin Flynn | *USEPA*

The Proposed Tier 2 Medaka Extended One-Generation Reproduction Test (MEOGRT)

Additional Authors: K. Flynn, D. Hammermeister, D. Lothenbach, USEPA NHEERL MED; J Swintek, Environmental Management Resources; F Whiteman, USEPA NHEERL MED

The Food Quality Protection Act of 1996 requires USEPA to develop and implement a program using valid tests for determining the potential endocrine effects from pesticides. The USEPA established advisory group, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), recommended USEPA develop a two-tiered approach: Tier 1 would identify the potential of a substance to interact with the endocrine system whereas Tier 2 would further identify and characterize chemical-induced interactions with estrogen, androgen and thyroid hormones for risk assessment to inform regulatory decisions. One of the Tier 2 tests being developed by the USEPA is a fish test that encompasses critical life stages and allows for the potential that the endocrine disruption could be expressed later in life, with particular interest on the reproductive period. This test will used to evaluate the adverse consequences of putative endocrine disrupting chemicals, especially those active within the hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPG) systems, on the development, growth and reproduction of fish. The MEOGRT is intended to serve as a higher tier test with a fish for collecting definitive concentration-response information on adverse effects suitable for use in ecological risk assessment. The general experimental design entails medaka (*Oryzias latipes*) to five different concentrations of a test chemical and a control over two generations with an optional third generation possible. The exposure starts with F0 adults, continues through the entire lifecycle of the F1 generation, and either concludes with hatch of the offspring from F1 or another complete lifecycle of the F2 generation. There are six replicates in each test concentration and 12 replicates for the control until the reproductive assessment at which time the replication structure is doubled to 12 replicates in each test concentration and 24 replicates for control. Endpoints evaluated during the course of the exposure include those indicative of generalized toxicity, i.e., mortality, abnormal behavior, pathology and growth determinations (length and weight), endpoints designed to characterize specific endocrine toxicity modes of action targeting estrogen (E)-, androgen (A)-, or possibly thyroid (T)-mediated pathways, and finally fecundity, a population relevant measurement.

Sigmund Degitz | *USEPA*

Development of the Larval Amphibian Growth and Development Assay (LAGDA)

Additional Authors: J.T. Haselman

The Food Quality Protection Act of 1996 requires USEPA to develop and implement a program using valid tests for determining the potential endocrine effects from pesticides. The USEPA established advisory group, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), recommended USEPA develop a two-tiered approach: Tier 1 would identify the potential of a substance to interact with the endocrine system whereas Tier 2 would further identify and characterize chemical-induced interactions with estrogen, androgen and thyroid hormones for risk assessment to inform regulatory decisions. One of the Tier 2 tests recommended by EDSTAC is an amphibian full life cycle test to evaluate the adverse consequences of putative endocrine disrupting chemicals, especially those active within the hypothalamic-pituitary-thyroid (HPT) and hypothalamic-pituitary-gonadal (HPG) systems, on the development, growth and reproduction of amphibians (specifically the frog). The Larval Amphibian Growth and Development Assay (LAGDA) is intended to serve as a higher tier test with an amphibian for collecting definitive concentration-response information on adverse effects suitable for use in ecological risk assessment. Specifically, the design enables the collection of amphibian hormone-regulated endpoint data (e.g., metamorphosis, gonadal development) and information concerning various aspects of the reproductive biology and life-stage viability. The general experimental design entails exposing Nieuwkoop Faber (NF) stage 8 X. laevis embryos to four different concentrations of a test chemical and a control until 10 weeks after the median time to completion of metamorphosis (NF stage 62) in the control with one interim sub-sample at NF stage 62 (See Nieuwkoop and Faber 1994 for staging details). There are four replicates in each test concentration with eight replicates for the control. Endpoints evaluated during the course of the exposure include those indicative of generalized toxicity, i.e., mortality, abnormal behavior, and growth determinations (length and weight), as well as endpoints designed to characterize specific endocrine toxicity modes of action targeting estrogen (E)-, androgen (A)-, or thyroid (T)-mediated pathways.

Tim Verslycke | *Gradient*

Validation of the Mysid Two-Generation Toxicity Test for the Regulatory Testing of Endocrine Active Compounds

Additional Authors: D. Mayfield, Gradient; V. Brown, D. Houchens, Battelle; P. Browne, L. Touart, US Environmental Protection Agency, Office of Science Coordination and Policy (OSCP)

USEPA developed the Endocrine Disruptor Screening Program (EDSP) in response to a US Congressional mandate "to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other effects as [USEPA] may designate" [21 U.S.C. 346a(p)]. As part of the EDSP, USEPA is validating assays to identify and characterize the endocrine activity of pesticides, commercial chemicals, and

environmental contaminants, specifically in relation to estrogen, androgen, and thyroid hormones. USEPA is also working with the OECD's Endocrine Testing and Assessment Advisory Group to validate and harmonize endocrine screening tests of international interest. Invertebrates (especially arthropods such as insects and crustaceans) constitute the vast majority of animal species on earth. Many invertebrate toxicity test protocols are routinely used in regulatory testing; however, few have been designed with endocrine-specific endpoints in mind. As part of the EDSP, a two-generation mysid toxicity test is proposed to provide a comprehensive Tier 2 evaluation of substances identified as possible endocrine disruptors through Tier 1 screening. Although the focus of the EDSP Tier 1 screening is on vertebrate relevant endocrine processes (estrogen, androgen, and thyroid), the mysid Tier 2 test is deemed necessary to assess potential adverse consequences of Tier 1 possible endocrine disruptors on invertebrate endocrine regulated processes. This talk will present a brief historical summary of the development and validation of a standardized mysid two-generation toxicity test proposed as an in vivo Tier 2 EDSP test. In addition, conclusions regarding strengths and weaknesses of the test in its current form will be presented.

Mary Ann Ottinger | *University of Maryland*

The Tier 2 Japanese Quail (*Coturnix japonica*) Avian Toxicity Test

The purpose of the Tier 2 Japanese Quail (*Coturnix japonica*) Avian Toxicity Test is to detect both short and long term impacts from exposure to Endocrine Disrupting Chemicals (EDCs). The Japanese quail is a precocial bird that has advantages of being a relatively domesticated, rapidly maturing, easily maintained, and well-characterized avian model. Studies have been conducted to inform the design of a avian two-generation testing protocol and the ascertain key measurement endpoints that provide reliable indicators of EDC exposure. These studies have included egg injection and several types of dietary studies that have considered a range of compounds. Egg injection studies take advantage of avian embryonic development in the egg, independent of parental input. As such, egg injection studies mimic maternal deposition of chemicals, providing an opportunity to dose the embryo with known concentrations of compound and track effects throughout ontogeny. Findings from comparison of existing studies reveal that many EDCs do impact avian species in support of observations of wild populations. A brief overview of impacts from various classes of EDCs will be provided. These studies also emphasize the unique characteristics of avian species, which must be considered by a testing protocol, including high body temperature, migration associated energy demands, precocial and altricial birds, high metabolic rate, and mechanisms and role of steroid hormones in sexual differentiation. In addition, potential sources of variability occur due to strain differences and between species relative to sensitivity to EDCs. Analyzing core endpoints of survival, growth, and reproduction across generations will reveal potential impacts on reproductive, metabolic/thyroid systems, and adrenal/stress axes as well as general toxicity. Each generation provides unique information and insights, specifically the parent generation reveals response in adults, the F1 generation is exposed through maternal deposition and the diet, and the F2 generation reveals impacts solely from embryonic exposure. It is important to assess measurement end points reflective of neural mechanisms regulating reproductive endocrine function and behavioral response, metabolic and stress axis function, and functional measures indicative of adverse physiological outcomes. Finally, comparative studies provide the necessary information to assess the broad applicability of findings from two generation studies in Japanese quail to other avian species. This is important for future applications that will use the data generated to assess risk across the wide range of breeding strategies and diversity of life histories with consideration of sensitivity and period(s) of vulnerability in order to protect avian populations.

Allen W. Olmstead | *Bayer CropScience*

Tier 2 EDSP Assays Viewed Through the Lens of Ecological Risk Assessment

Ecological risk assessment is the process through which the likelihood that adverse effects in the environment occur due to a stressor. Generally for chemical substances, assessments are made at the level of the individual on processes of survival, growth, and reproduction with the assumption that by safeguarding these, populations would be protected from adverse effects. Endocrine toxicity represents one of many means through which these processes may be affected. While currently a large battery of ecotoxicology tests are employed to assess the hazard, these are not tailored specifically to endocrine toxicity. The EDSP should evaluate hazard that is not covered by current testing. The tier II EDSP assays should be evaluated based on what additional hazard information that has a meaningful impact on ecological risk assessment is generated beyond that from current test guidelines. Further, the endpoints measured in these test should be optimized with respect to their utility in evaluating ecological risk.

Henry Krueger | *Wildlife International, Division of EAG*

Contract Laboratory Perspective on Higher-Tier Endocrine Testing

Additional Authors: T. Springer, S. Gallagher, S. Schneider, Wildlife International, Division of EAG

Tier 1 EDSP testing is well underway and test methodologies for Tier 2 tests have been developed and validated, with guidelines near completion. While we are further down the road and about to confront the beginning of Tier 2 endocrine testing in contract labs, questions and concerns remain. For contract labs and all stake holders, the real experiment(s) is about to begin and the size and complexity of the Tier 2 experimental designs provide challenges for us all. An important question is how we incorporate the information we have from Tier 1 and other tests in designing Tier 2 tests. Do we have enough information to design and run Tier 2 tests? More basic questions from the contract lab perspective concerns how we select test concentrations for Tier 2 endpoints and how we deliver test materials to achieve those concentrations. One of the toughest problems in selecting test concentrations is making sure that the upper limit of testing levels are set at maximum tolerated doses so that endocrine effects are not confounded by effects caused by general or systemic toxicity. Determining the maximum tolerated dose will be difficult, since there will be data gaps for several of the Tier 2 endpoints. A good example of this is the lack of data on the effects of maternal transfer and in ova exposure in the Medaka Tier 2 fish test. Another consideration is the lack of data for fish endocrine endpoints like VTG, fecundity, and secondary sex characteristics over lifetime exposures. Perhaps there is room for what have been described as Tier 1.5 studies that may provide an opportunity to collect more definitive information to be used in designing Tier 2 tests. These intermediate tests may even provide data that preclude the need for additional testing. With the large number of chemicals that need to be tested and the limited capacity of contract laboratories, identifying the potential for endocrine disruption at an intermediate stage not only makes good sense, but also is good science.

Peter Matthiessen | *Independent Consulting Ecotoxicologist*

Perspectives on Hazard and Risk-based Approaches to the Evaluation of Endocrine Disrupting Chemicals

Although the European Union has still not finalised a regulatory definition of endocrine disrupting chemicals (EDC), legislation already in place mandates European regulators to withdraw a pesticide or biocide from the market if it has been identified as belonging to this group of substances, and a roughly similar line will be taken with industrial chemicals. In essence, this approach solely considers the hazards which an EDC may pose, and does not calculate risk. This contrasts with the methods expected to be followed elsewhere (e.g. US; Japan) which are risk-based. The reasons for this fundamental difference need to be understood, and it is of course not unheard of for certain groups of chemicals to be regulated by their hazard alone – for example, highly persistent and bioaccumulative substances listed under the POPS Convention are automatically banned around the world for most applications, as are genotoxic carcinogens. The motivations for regulation by hazard alone are simple – thus, it is used as a precautionary measure in circumstances where it is felt that risk assessment is unreliable due to uncertain exposure (e.g. POPS), or where no safe dose or concentration can be estimated (e.g. genotoxic carcinogens). The argument has been made in the EU that safe concentrations/doses of EDCs cannot be calculated due to the alleged absence of toxic thresholds and the claimed prevalence of non-monotonic dose-responses. The question which has still not been satisfactorily resolved is whether these alleged phenomena are real, or of sufficient importance to justify the economically serious decision to ban EDCs because of their properties alone. This presentation will explore these issues.

Holly Zahner | *US Food and Drug Administration*
Jane Staveley | *Exponent*

Regulatory Approaches to Evaluation of Endocrine Disrupting Compounds at Various Government Agencies

Additional Author: K. Yamazaki, Ministry of the Environment Japan

This presentation will discuss the activities of US federal regulatory agencies other than the USEPA, which administers the Endocrine Disruptor Screening Program (EDSP), as well as provide a brief overview of current activities in Canada and Japan. The US Food and Drug Administration (USFDA) evaluates the potential for environmental impacts from the use of endocrine disrupting compounds (e.g., steroid hormones) in human and veterinary pharmaceuticals under the National Environmental Policy Act. The current environmental risk assessment approaches used at USFDA's Center for Veterinary Medicine (CVM) and Center for Drug Evaluation and Research (CDER) to evaluate the potential risks of animal and human drugs containing steroid hormones will be presented. CVM has developed a targeted approach that uses appropriate science to evaluate the potential exposures and effects from steroid hormones used in drugs administered to livestock, such as hormone implants. An overview of CDER's work on a draft guidance for industry on the nonclinical evaluation of endocrine disruption potential of drugs (Endocrine Disruption Potential of Drugs: Nonclinical Evaluation) will also be presented. Under the Canadian Environmental Protection Act, which requires that all substances in Canadian commerce be evaluated for potential risks to the environment and human health, a framework has been developed for evaluating active pharmaceutical ingredients, including hormones. In Japan, the Ministry of the Environment has developed the EXTEND program to assess the environmental risk of endocrine disrupting substances. This program involves the development of test methods and an assessment framework, effects assessment of selected chemicals (to include testing, if necessary, in a tiered process) detected in the aquatic environment and risk assessment/management, as well as the promotion of research.

Katherine Coady | *The Dow Chemical Company*

Octylphenol and Nonylphenol as Case Studies for Determining the Relevance of the Endocrine Mode of Action in Human Health and Environmental Assessments

Additional Authors: J. Klapacz, The Dow Chemical Company; T. Osmitz, Science Strategies; C. Staples, Assessment Technologies; B. Losey, RegNet Environmental Services

Efforts to develop approaches and guidelines for the assessment of endocrine active compounds are now underway in various regions. Nonylphenol (NP) and octylphenol (OP) are data rich chemicals that provide an opportunity to examine the relevance of the endocrine mode of action to human health and environmental assessments. Systematic assessment of the potential endocrine activity of NP and OP in well-conducted screening-level studies (in vitro and in vivo) confirm that these compounds exhibit potential endocrine activity in the hypothalamus-pituitary-gonadal axis. NP and OP have weak estrogenic-like activity based on various in vitro studies with the estrogen receptor with potencies generally ranging from 1,000 to 1,000,000-fold weaker than the endogenous estrogen, estradiol. However, multiple lines of evidence indicate that these alkylphenols have other relevant modes of action in biological systems apart from just weak endocrine activity. Closer assessment of the toxicological data sets for NP and OP demonstrate that other modes of action, apart from endocrine activity, are influential in both human health and environmental assessment of these endocrine active compounds. This is apparent when examining the effects in higher-tiered, definitive toxicity tests (chronic, multigenerational in vivo studies with apical endpoints) which indicate that the weak endocrine activity of NP and OP does not predict the entire suite of observed adverse effects in both mammalian and non-mammalian systems. Particularly in the case of OP, the weak estrogenic mode of action does not appear to be the critical effect in higher tiered toxicity tests. Concentrations of these alkylphenols detected in human biomonitoring studies are orders of magnitude below levels of concern for human health, and the great majority of concentrations of NP and OP in the environment are below levels of concern for environmental organisms. These case studies with NP and OP illustrate the need to incorporate the concepts of potency, critical effect, exposure, and risk in decision-making regarding determinations of endocrine disruption and assessments of human health and environmental impacts.

Daniel J. Caldwell | *Johnson & Johnson*

Magnifying Perceived Risk: A Case Study of Hazard and Risk Assessment of a Pharmaceutical Compound, 17α-Ethinylestradiol (EE2)

Inaccurate or snapshot field measurements used as "environmentally relevant" test concentrations in laboratory studies, biomarker detection (i.e., vitellogenin in male fish) incorrectly reported as an effect, and field experiments using confined exposure (i.e., lake) being inappropriately extrapolated to surface water (river) risk assessment have contributed to the misconception that EE2 exposure is of great consequence to wildlife and humans. Hazard assessments using in vitro studies typically depict EE2 as a potent EDC. Using in vivo data, safe exposure levels for EE2 for aquatic species and humans were developed and a

sufficient Margin of Safety demonstrated for aquatic species exposed in surface waters, and for humans potentially exposed via drinking water. However, continued attention is directed to this compound, including imposition of specific monitoring requirements in Europe while ignoring the multitude of other estrogenic substances present in surface waters. A better approach is to establish a level of estrogenic activity that is without population impact and monitor waters for that endpoint. Estrogen-active substances are the ideal test-case for this approach for several reasons. First, they act by a common mechanism of action that has been shown to demonstrate concentration-addition effects, i.e., additivity. Second, there are multiple categories of estrogen-active substances, naturally produced estrogens, naturally produced phytoestrogens, synthetic estrogens (e.g., EE2), and industrial chemicals (e.g., phthalates, BPA, octyl phenol, nonyl phenol) that have demonstrated estrogenic activity. Comparing the relative differences in occurrence/concentration and the relative differences in estrogenic effect among these categories facilitates a science-based understanding of the relative importance of the individual substances to the total estrogenic load to which ecosystems, and potentially humans, are exposed. Monitoring data suggest that exposures of fish to EDC in surface water are largely due to chemicals other than EE2 and that observed effects are likely due to the total estrogenic load, of which EE2 is a minor contributor. Unless estrogenic activity of surface water is addressed holistically we may miss important contributors to the total estrogenic exposure by focusing on individual EDCs.

Steven L. Levine | *Monsanto Company*

Tier 1 Endocrine Disruptor Screening Program (EDSP) Assays and Regulatory Safety Studies Provide a Weight of Evidence that Glyphosate is not an Endocrine Disruptor

Glyphosate was in the initial group of compounds that received test orders to be under the USEPA's EDSP. The compounds in this initial group were selected based on qualitative exposure potential, including information from a range of historical monitoring data bases, and are not known or suspected to interact with the endocrine system. Ideally, under an assessment of potential endocrine effects, realistic considerations must be given to exposure and potency. These considerations can highlight the insignificance of exposure to very low potency substances. Although targeted in vitro and in vivo screens can provide useful information for hazard identification, the Tier 1 screening approach and data interpretation does not take into account realistic environmental exposures. In addition to a review of the recent Tier 1 EDSP results for glyphosate, this presentation will speak to concepts of potency, exposure, and risk in regulatory decision-making for assessments of human and environmental impacts of endocrine active compounds. Glyphosate was tested in all 11 Tier 1 screening assays although a significant amount of OSRI was available from regulatory studies and the open literature. In vitro assays showed glyphosate is not (anti)-estrogenic or (anti)-androgenic and does not impact steroidogenesis. These findings are fully consistent with peer-reviewed studies using functionally equivalent in vitro assays and predictions from SARs. Additionally, the Tier 1 in vitro results are consistent with the Tier 1 in vivo results from existing safety studies that provide information of the same nature. Higher Tier studies can provide evidence of endocrine disruption or lack thereof, and are viewed as powerful tools for hazard assessment. Consistent with the results from several multi-gen rat studies, glyphosate had no impact on estrogenic, androgenic, steroidogenic and thyrogenic endpoints below the MTD in in vivo mammalian assays, including the Uterotrophic, Hershberger, and male and female pubertal assays. Consistent with the pubertal assays, the AMA found no impact on thyroid-driven developmental endpoints. Results from the FSTRA study were consistent with the results of an existing fish full life cycle and showed no impact on all measured endpoints. From the weight of evidence provided by the Tier 1 assays along with the higher Tier regulatory safety studies, it can be concluded with a high level of confidence that glyphosate is not an endocrine disruptor.

L. Earl Gray Jr | *USEPA*

Nonmonotonic Dose Response Curves (NMDRCs) Are Common After Estrogen or Androgen Signaling Pathway Disruption. Fact or Falderal?

The shape of the dose response curve in the low dose region has been debated since the late 1940s. The debate originally focused on linear no threshold (LNT) vs threshold responses in the low dose range for cancer and noncancer related effects. Recently, claims have arisen that the endocrine disrupters (EDs), which act via high affinity, low capacity nuclear receptors, commonly induce effects displaying NMDRCs at low doses which would be missed in standard multigenerational toxicity studies. This presentation will discuss LNT, threshold and NMDRCs responses from case studies of chemicals that disrupt reproductive development and function via the androgen and estrogen signaling pathways and will include in vitro and in vivo multigenerational data. The in vivo studies in this discussion include only robust, well-designed, comprehensive studies that administered the chemical via a relevant route(s) of exposure over a broad dose response range, including low dose(s). The chemicals include ethinyl estradiol, estradiol, genistein, bisphenol a, trenbolone, finasteride, flutamide, phthalate esters, selective estrogen receptor modulators and inhibitors of aromatase. The objective is to critically evaluate the reproductive and developmental toxicity data from well-done studies in this field to address concerns that current multigenerational reproductive test guidelines are missing adverse low dose effects because EDs routinely induce nonmonotonic adverse low dose responses. My current conclusions are: 1) EDCs appear to induce some LNT effects. 2) NMDRCs are biologically plausible and occur frequently in vitro, but these often occur in vitro at high concentrations of estrogens or androgens that are not relevant in vivo. 3) It appears that NMRDCs are more common 3a) in short- versus long-term exposures and 3b) with upstream, mechanistic events versus downstream phenotypic effects. 4) A few adverse effects of EDs are non-monotonic, but other effects displaying monotonic responses occur at lower dosage levels. 5) A number of robust multigenerational studies of estrogens and antiandrogens have been executed and NMDRCs were uncommon at low dosage levels. 6) Multigenerational Test guidelines can be enhanced on a case-by-case basis to improve the sensitivity to low dose effects of some EDCs. 7) Additional data needs to be examined from robust, multigenerational studies using a broad range of dosage levels for other pathways. This is an abstract of a proposed presentation and does not necessarily reflect USEPA policy.

Christopher J. Borgert | *CEHT, University of Florida*

Modernizing Problem Formulation for Risk Assessment: Potency and Mass Action Govern Endocrine Activity

The questions posed (i.e., the hypotheses tested) by a scientific investigation determine the measurements taken and conditions under which they are evaluated, the procedures used to record and analyze data, and the context in which results are interpreted. In risk assessment, the questions addressed are typically articulated in the problem formulation phase, which includes hazard identification (HI). However, HI procedures were formulated to address questions involving overtly observable

adverse effects, e.g., acute toxicity, cancer and reproduction, in an era when mechanistic understanding was scant. Thus, HI processes do not address the types of mechanistic issues that arise in identifying endocrine activity, and unlike basic sciences, have not been modernized to keep pace with advancements in biological and pharmacological understanding. Vital signaling functions of the endocrine system require it to continuously discriminate the biological information conveyed by potent endogenous hormones from a more concentrated background of structurally similar, endogenous molecules with low hormonal potential. This obligatory ability to discriminate important hormonal signals from background noise is achieved through differential potency and laws of mass action, which can be used to define thresholds for induction of hormonal effects. Without such thresholds, normal physiological functions would be impossible. The fundamental principles governing hormonal effects – affinity, efficacy, potency, and mass action – dictate the existence of thresholds for hormonal activity and also define the potential that exogenous chemicals might have to interfere with normal endocrine functioning. Assessment of these properties are well established in endocrine pharmacology, but have not yet been incorporated into HI for risk assessment. For risk assessments of endocrine active substances to claim a basis in modern science, the problem formulation phase and the HI process must be modernized to address potency and thresholds for endocrine activity.

Gerald A. LeBlanc | *North Carolina State University*

Emerging Targets of Endocrine Disruption: The Xenocrine System

The 2012 OECD monograph State of the Science on Novel In Vitro and In Vivo Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors undertook the formidable task of identifying the wealth of endocrine signaling processes that have known susceptibility to disruption by environmental chemicals and assays that can be used to evaluate such disruption. Among the pathways recommended for future priority consideration were the PPAR, vitamin D, thyroid, and retinoid signaling pathways. Noteworthy, is that only one of these pathways utilizes a classical hormone (thyroid hormone pathway). The remaining three involve ligands of exogenous origin and related to diet (i.e., xeno signaling molecules: vitamins, fatty acids). Furthermore, all four pathways utilize the retinoid X-receptor (RXR) as a partner receptor in the signaling process. Taken together, these signaling pathways provide a coordinated network that links energy acquisition and utilization. Disruption of this network can have implications on various endemic disease conditions in the human population (e.g., obesity, diabetes, metabolic syndrome) and energy intensive processes in wildlife (e.g., growth, reproduction). Targets of disruption within this network include xeno-agonists/antagonists of the receptors, xeno-agonists/antagonists of the RXR, modulators of multi-functional coactivators, and modulators of intracellular receptor levels. Possible modes by which environmentally relevant exposure levels of chemical mixtures might elicit additive or synergistic effects on the network are discussed.

Seth W. Kullman | *North Carolina State University*

Emerging Technologies to Assess Endocrine Disruption: Overview of New Assays and Approaches that Show Promise in Evaluating the Endocrine Activity of Chemicals

The USEPA and the Organization for Economic Cooperative Development (OECD) have cooperatively established test guidelines to detect endocrine disruptors (EDCs) and to harmonize risk characterization approaches for EDCs. These guidelines have been integrated into a Conceptual Framework that can be used to evaluate chemicals for endocrine-disrupting activity with a focus on estrogen, androgen, and thyroid (EAT) signaling processes. While, the EAT pathways represent three prominent endocrine pathways, recent evidence indicates that other endocrine pathways are additionally susceptible to the disrupting effects of environmental chemicals. Recently a detailed review (DRP) paper was drafted that describes select assays used throughout the research community to detect endocrine-disrupting effects of chemicals on non-EAT pathways, atypical EAT pathways (e.g., estrogen signaling via membrane receptors), and neuro-endocrine pathways. Here I discuss the overall intent of the DRP as a mechanism to provide guidance on testing approaches that can be used for assessing the actions and toxicity of chemicals on endocrine pathways not addressed in current Test Guidelines. Additionally the USEPA has begun to establish Adverse Outcome Pathways to link molecular initiating events with apical outcomes of EDC exposures. The goal of this discussion is to exploit our current knowledge of molecular initiating events (MIEs) and chemical mechanism(s) of action to identify novel in vitro and/or in vivo approaches to screen for endocrine disrupting activities. Mechanisms and assays presented are provided in the context of establishing AOPs to further the mission of identifying new pathways for endocrine disruption and facilitate linkage of MIEs and apical endpoints with robust detection of EDCs.

Kevin M. Crofton | *USEPA*

Using High-Throughput Methods to Conduct Risk-based Prioritization of Chemicals for the EDSP

Additional Authors: R. Judson, R.S. Thomas, National Center for Computational Toxicology, USEPA

A major challenge for regulatory authorities is how to assess risk for the large universe of untested chemicals for which there is human or ecological exposure. This chemical universe has been estimated to be 30K-50K unique substances. In particular, the USEPA Endocrine Disruption Screening Program (EDSP) is required to test 5-10K chemicals due to pesticidal use or their potential to contaminate drinking water. The USEPA Computational Toxicology program is developing methods and models to prioritize this large chemical universe for further testing based on estimation of risk. The prioritization framework includes consideration of hazard, exposure and dosimetry. Hazard estimation combines in vitro high-throughput screening (HTS) assays plus QSAR and docking models. The USEPA's ToxCast program, together with the US Interagency Tox21 program, has generated data for over 8500 chemicals using a variety of HTS assays for endocrine activity (e.g., ER, AR, TR). Additionally, a large-scale multinational effort is developing and evaluating QSAR and docking models to use and extend the HTS data, initially for the estrogen receptor. Results of this modeling effort will in turn drive further in vitro testing. USEPA's ExpoCast program is developing quantitative exposure prediction models based on chemical properties and use patterns. These models allow rapid estimates of exposure potential for thousands of chemicals. Finally, for dosimetry, we are using a combined in vitro and modeling approach (called RTK or Reverse Toxicokinetics) to provide quantitative estimates of dose-to-concentration scaling. By combining quantitative in vitro potency estimates from ToxCast, concentration-to-dose scaling from RTK, quantitative exposure values from ExpoCast, and estimates of uncertainty, we can provide quantitative risk metrics at the pathway level (estrogen, androgen, thyroid) for hundreds to thousands of chemicals. The first use of these estimates will be in prioritizing chemicals for inclusion in the EDSP Tier 1 assay battery. This abstract does not necessarily reflect Agency policy.

Patricia Schmieder | *USEPA*

Developing In Silico Approaches to Chemical Prioritization for ED Testing within an AOP Context

Additional Authors: M. Hornung, R. Kolanczyk, M. Tapper, J. Serrano, J. Denny, USEPA,ORD, NHEERL, Mid-continent Ecology Division

The USEPA, ORD aquatic toxicology lab in Duluth has been developing predictive toxicity models for use in risk assessment of data poor industrial chemicals since the 1980s. More recently a QSAR-based expert system was developed to prioritize among thousands of chemicals the USEPA must consider for EDSP testing. An ER Expert System was developed demonstrating how a chemical prioritization tool is developed based on early events plausibly connected to adverse outcomes of risk concern. The use of the OECD QSAR validation principles guiding the development of the system to be transparent and useful for the intended regulatory purpose will be presented. How the approach can be used to guide development of predictive models where other adverse outcome pathways have been sufficiently elucidated will be discussed.

Miriam N. Jacobs | *Public Health England*

In Vitro and In Vivo Testing Methods of Epigenomic Endpoints for Evaluating Endocrine Disruptors

Additional Authors: J.M. Greally, Center for Epigenomics, Albert Einstein College of Medicine

Epigenetic modulations underlie critical developmental processes and contribute to determining adult phenotype. Alterations to the phenotype, due to exposure to environmental insults during sensitive periods of development, are mediated through alterations in epigenetic programming in affected tissues. Originally prepared for the Organisation of Economic Cooperation and Development (OECD), this detailed review evaluates the potential role of chemical-induced epigenetic modifications to endocrine signaling pathways during sensitive windows of exposure as a mechanism of endocrine disruption, along with the examination of potential methods for assessing such disruption. Potential targets of disruption along putative adverse outcome pathways associated with the signaling pathways are identified, along with assays that show promise in evaluating the target in a screening and testing program such that in vitro methods are used where possible, and animal experiments only where in vitro methods are not available. Monitoring such epigenetic marks in response to toxicant exposure may in future provide a valuable tool for predicting adverse outcomes, but a more robust basis for Test Guideline recommendations is still needed. Although there is evidence to suggest that epigenomic dysregulation might mediate effects of exposures to endocrine disruptors, it is uncertain as to whether these changes are truly predictive of adverse outcome(s). Adverse effects observed in the OECD transgenerational assays could be used to inform future tests specifically designed to investigate the epigenetic mechanism of action. Follow-up studies should include both an epigenetic as well as a genomic component to differentiate between the contributions of potentially compensatory mechanisms.

Amy E. Yahnke | *University of Washington*

1 Imazapyr and Listed Amphibians: Applying Phenology to Toxicity Testing

C. Grue, A. Troiano, Washington Cooperative Fish and Wildlife Research Unit, School of Aquatic and Fishery Sciences, University of Washington; M. Hayes, Science Division, Habitat Program, Washington Dept of Fish and Wildlife

Conflict between native amphibians and aquatic weed management in the Pacific Northwest is rarely recognized because most native stillwater-breeding amphibian species move into upland habitats during the summer, when application of herbicides to control weeds in aquatic habitats typically occurs. However, for aquatic species present in wetland habitats through the summer, such as the Washington State Endangered Oregon Spotted Frog (*Rana pretiosa*) and the larvae of selected salamanders, aquatic weed management may pose a risk. Acute toxicity of herbicides used to control aquatic weeds tends to be low, but the direct effects of herbicide tank mixes (active ingredient, surfactant, and dye) on Oregon Spotted Frogs has remained unexamined. To address this gap, we exposed juvenile Oregon Spotted Frogs to mixtures of the herbicide imazapyr (Polaris AQ, 28.7% active ingredient), Agri-Dex surfactant, and Hi-Light dye in a 96-hour static-renewal test. The imazapyr-Agri-Dex combination was chosen because of its low toxicity to fishes and its preference by state land managers for aquatic weed control. Concentrations were those associated with low (3.5 liters/hectare) and high (7.0 liters/hectare) volume applications of Polaris AQ to control Reed Canarygrass (*Phalaris arundinacea*), and a clean-water control. Following exposure, frogs were reared for 2 months in clean water to identify potential latent effects on growth. Endpoints evaluated included feeding behavior during the exposure and grow-out, growth, and liver condition index. We recorded no mortalities. We also found no significant differences for any endpoint between the herbicide-exposed and clean-water control frogs.

Breanne E. Holmes | *University of North Carolina*

2 Disinfection Byproducts and the Endocrine System: Evaluating the Affinity of Low-Level Environmental Contaminants

L. Smeester, R. Fry, H. Weinberg, University of North Carolina

The process of preparing drinking water supplies for human consumption includes the use of strong oxidizing disinfectants to inactivate microbial contamination. While disinfection helps to prevent waterborne illnesses, it also leads to the formation of a class of compounds known as disinfection byproducts (DBPs). The health effects of these chemicals, which result from the reaction of disinfectants with organic and inorganic compounds already present in the water source, are an ongoing area of research. During the past 35 years, a variety of toxicological studies in rodents showed a potential association of some DBPs with a number of cancers, including those of the liver, kidney, and large intestine, as well as with developmental and reproductive effects. The influence of these chemicals on the endocrine system, however, is not fully understood. The health effects on humans through drinking water consumption are of particular interest, because this is a potential source for chronic low-level exposures. In order to elucidate whether DBPs can play a role in endocrine disruption, 21 individual species were tested in both the estrogen and androgen receptor competitive binding assays. To determine their binding affinity, they were evaluated in concentrations ranging from 0.1 nM to 2 mM in each assay, against a static concentration of tritium-labeled reporter ligand. A comparison between each of the compound activities in either the estrogen or androgen receptor assays was then determined and structural comparisons between effective compounds were studied. The strongest binding affinities, as measured by their EC50 values, were found for bromine-, chlorine-, and phenol-containing compounds.

Charles B. Tate | *Appalachian State University*

3 Differential Binding of Land Applied Wastewater to the Three Estrogen Receptors: ERa, ERba, and ERbb of the Atlantic Croaker (*Micropogonias undulatus*)

M.B. Hawkins, Dept of Biology, North Carolina State University; E. Nichols, Dept of Forestry, North Carolina State University

The binding of estrogens to estrogen receptors (ERs) in target tissues plays a key role in the regulation of many important physiological processes. Xenoestrogens are natural and synthetic chemicals that are released into the environment and can bind to and activate ERs. These endocrine-disrupting compounds (EDCs) can make their way into aquatic ecosystems, which may lead to the disruption of normal endocrine function in fish and other species. Due to the prevalence of EDCs in the environment and their potential to harm wildlife, it is important to develop an effective screening assay that encompasses a broad range of possible estrogenic compounds. Although vertebrates possess multiple ER subtypes with different ligand binding and transactivation properties, most screening is done using a single ER subtype, the human ERa. This approach may result in an underreporting of estrogenic contamination, particularly for non-mammalian species. Teleost fish express three ER subtypes (ERa, ERba and ERbb), with ligand binding profiles distinct from each other and mammalian ERs (ERa and ERb). We used bacterially expressed teleost ERs in competitive binding assays to investigate the ability of groundwater samples from a municipal site that uses tree spraying for wastewater treatment to bind ERs. Groundwater collected from Well 3 at the site was concentrated by vacuum centrifugation (SpeedVac) and reconstituted at 10X concentration. Well 3 water concentrate had a higher relative binding affinity (RBA) to the ERs than did controls at 1X, 3X, and 6X concentrations. However, the RBAs were not dose dependent, suggesting assay inhibition at higher concentrations.

Clifford Habig | *Compliance Services International*

4 Experiences with Test Concentration Selection for FSTRA and AMA Testing: What Has Worked and What Has Not Worked

USEPA has provided very detailed guidance documents for the Fish Sort-Term Reproduction Assay (FSTRA) and Amphibian Metamorphosis Assay (AMA). This guidance includes recommendations for selecting the three test concentrations required for each assay. For both assays, the highest test concentration should be just below the maximum tolerated concentration (MTC), with either the water solubility limit or 100 mg a.i./L being the limit concentration for testing. The MTC represents a concentration just below the highest achievable concentration that does not result in significant toxicity (<10%) to the test organisms. We have tested approximately six organophosphorus (OP) insecticides and two to three non-OP pesticides

that were included on USEPA's list 1 for Tier 1 testing. Even among the OP compounds, these test substances have varying physio-chemical properties. For both the FSTRA and AMA studies, the test guidelines recommend a high test concentration of one-third of the fish 96-hour LC50 value as an estimate for the MTC. Preferably, the 96-hour LC50 should be on the same species as the species used in the FSTRA study (fathead minnows) and the AMA study (*Xenopus* tadpoles). Discussion will focus on our experiences with selecting test concentrations to meet USEPA's criteria. Our experiences, indicate that using one-third of the 96-hour LC50 as the highest test concentration, even when conducted on the same species and life stage as the animals used for FSTRA or AMA testing, has resulted in unreliable results. Depending on the test compound, using a high concentration of one-third of the LC50 has resulted in a high test concentration that is either too high, resulting in toxicity to the test organisms, or a concentration that is too low. These results will be discussed, and may be related to properties of the test substances. We will also discuss results using subchronic data (fish early life stage) data to estimate an MTC for the FSTRA test, as well as unexpected water solubility issues based on previous data. Discussion will also include experiences with selecting concentrations for the AMA test. Overall, we have obtained the most reliable results for selecting test concentrations by conducting 14 to 15-day range-find studies using the same life stage as organisms tested in the FSTRA and AMA studies.

David R. Luthin | *McWhorter School of Pharmacy, Samford University*

5 Quantification of Estrogen Analytes in the Discharge from Water Treatment Plants (WWTPs) in Jefferson County, Alabama

L.Coward, McWhorter School of Pharmacy, Samford University; B. Arwood, Dept of Biology, University of Alabama Birmingham; K. Palmore, P. Carpenter, K. O'Neil, McWhorter School of Pharmacy, Samford University; R. Angus, Dept of Biology, University of Alabama Birmingham; G. Gorman, McWhorter School of Pharmacy, Samford University

Natural and synthetic estrogens are some of the most potent endocrine disrupting compounds (EDCs) in the aquatic environment that can cause changes in sexual and reproductive characteristics of fish and potentially have effects on humans. The five most common analytes include estrone (E1), 17-β-estradiol (βE2), Estriol (E3), 17-α-estradiol (αE2) and the synthetic agent 17-α-ethinylestradiol (EE2). We quantified these 5 analytes by two different methods using triple-quad LC-MS in wastewater effluent from six different WWTPs in Jefferson County near Birmingham, AL. One L "grab" samples at mid-stream depth of river water were collected 50 m upstream, 50 m downstream and directly from the site of discharge. Samples were filtered and then loaded onto solid phase extraction (SPE) Bond Elut cartridges, washed with 40% MeOH, then water and eluted with 10% MeOH in MTBE. In one protocol, an extra wash step was added using 10% MeOH/2% ammonia in water. Samples were dried under N2 and either reconstituted in LC-MS mobile phase (P1) or NaHCO3 buffer (P2). Water samples processed using the initial protocol (P1) produced high backgrounds which decreased the sensitivity of the method and made quantitation difficult at the low levels needed. In the revised protocol (P2) samples were then conjugated using a dansyl chloride solution, extracted with TBME and then dried under N2. P2 samples were dissolved in 80% Acetonitrile and analyzed by LC-MS. The dansyl chloride dervitization protocol was at least 10 fold more sensitive than the original and was therefore used to further quantify the river water samples. Presence of Estriol and Estrone were detected in effluent and downstream samples from one site only (2.93 and 0.664 ng/L, Estriol and 1.47 and 0.595 ng/L Estrone from effluent and downstream, respectively), with the other analytes not detected at any of the other sites tested. The impact on fish morphology of low levels of estrogen analytes present in the watershed is currently being investigated.

Gerald A. LeBlanc | *North Carolina State University*

6 High Throughput Assays to Assess Interactive Effects of Chemicals on Endocrine Signaling Pathways

L. An, S. Ectcheson, C. Holmes, G. Kwon, E. Medlock, Y. Wang, Environmental and Molecular Toxicology Program, Dept of Biological Sciences, North Carolina State University

The high-throughput evaluation of toxicity pathways is an emerging paradigm for future toxicity characterization. We are developing a battery of assays that incorporate both bioluminescence resonance energy transfer and transcription reporter assay technology to assess the ability of chemical combinations to target various components of an endocrine signaling pathway that may result in synergistic or other interactions. Two groups of signaling pathways are currently the focus of our research: the PPAR/RXR signaling pathways that contribute to lipid homeostasis and metabolic syndrome and the ecdysteroid/methyl farnesoate signaling pathways that regulate various aspects of growth, development, and reproduction in crustaceans. The technology involves cell-based assays in which proteins of interest are tagged with either a photon donor (Renilla luciferase 2) or a fluorescent photon acceptor (m-ametrine, EBFP2). The ability of receptor subunits (e.g. PPAR and RXR; EcR and RXR, MfR and SRC) to dimerize or dissociate in response to ligand is then evaluated by the fluorescence output upon dimerization or dissociation. For example, we have observed that the PPARα:RXR heterodimer forms spontaneously and the complex is then ligand activated to stimulate gene transcription. In contrast, formation of the RXR:RXR homodimer is ligand (e.g. tributyltin) dependent and this activated homodimer is then capable of stimulating gene transcription. Further, we have observed additive activation of PPARα:RXR in the presence of ligands to each subunit (e.g., diethylhexyl phthalate and tributyltin). Results demonstrate the utility of the approach for evaluating, and predicting, interactions among chemicals on endocrine signaling pathways.

Heather Gall | *Penn State*

7 Assessing the Presence and Impact of Endocrine Disrupting Chemicals in Vernal Pools

T. Langkilde, Dept of Biology, Penn State; O. Mina, Dept of Agricultural and Biological Engineering, Penn State; B. Carlson, Dept of Biology, Penn State

The land-application of pesticides and human and animal wastes to agricultural fields introduces endocrine disrupting compounds (EDCs) into the environment. Vernal pools near agricultural fields receive runoff likely containing EDCs, potentially causing adverse impacts to amphibians. Although some laboratory studies have been conducted to understand the impact of controlled exposure of amphibians to EDCs, no data exist for the occurrence of EDCs in vernal pools. We collected preliminary water quality data from 7 vernal pools in Central Pennsylvania that receive surface runoff from agricultural fields. Three pools are within a catchment that receives wastewater effluent irrigation, one is within a catchment that receives dairy manure applications, and three are in agricultural areas with unknown pesticide and fertilizer applications. The data presented at this meeting will include the baseline data collected in October, when the water level in the pools was very low, and samples collected later in the fall when the pools became filled. Samples are analyzed for 7 estrogens of human and livestock origin, 24 pesticides and their metabolites, nutrients (nitrogen, phosphorus, and carbon), and pH.

Water quality data collected from these sites will be used to design mesocosm studies in which tadpoles are exposed to controlled, environmentally relevant EDC concentrations. This novel study confronts three major challenges that must be overcome for it to succeed: (1) water samples contain sediments and organics, which make analysis difficult; (2) the short half-lives of hormones makes controlled exposure studies challenging; and (3) synergistic effects of EDCs poses challenges for interpretation and generalization of our results.

Heiko L. Schoenfuss | *St. Cloud State University*

8 A Multi-tiered Approach to Assessing Biological Effects of Contaminants of Emerging Concern on Fishes in Great Lakes Tributaries

L.M. Thomas, St. Cloud State University; Z.G. Jorgenson, US Fish and Wildlife Service; S.E. Bartell, Normandale Community College; K.E. Lee, S.J.Choy, J.N. Moore, J.A. Banda, D.J. Gefell, US Fish and Wildlife Service

There is growing concern over the presence, distribution, and possible biological effects of Contaminants of Emerging Concern (CECs) in the Great Lakes. To assess the risks of CECs on aquatic wildlife, the St. Cloud State University Aquatic Toxicology Laboratory is collaborating with the US Fish and Wildlife Service in collecting, analyzing and interpreting fish samples across several Great Lakes tributaries. This study will provide feedback to laboratory scientists relating the connectivity of laboratory-based effects assessments with environmentally based effects monitoring. In the first year of this study, we examined resident fish populations across three Great Lakes rivers and three sites within each river for physiological indications of exposure to endocrine disrupting compounds and alterations to reproductive health. In conjunction with resident fish collections, we deployed caged sunfish for 14 days at five to six sites within each river to gather more controlled exposure data and help in the determination of CEC sources. Finally, water and sediment samples were collected to assess presence and concentrations of a suite of contaminants of emerging concern. Resident (n=608) and caged (n=615) fish were assessed for a range of biomarkers including indications of morphometric, physiological and pathological changes to fishes consistent with exposure to estrogenic CECs. Enzyme-linked immune-assays (ELISAs) were used to quantify plasma vitellogenin concentrations in four fish species (white sucker, smallmouth bass, largemouth bass, sunfish) and histological assessments determined reproductive status and presence of pathologies in gonadal and liver tissues. To provide greater control in assessing the effects of CECs on fish health, we applied a behavioral assay previously shown to be sensitive to CEC exposure in larval fathead minnows. Larvae were exposed to field site-specific water (50% daily static renewal) for 21 days. Following the 21-day exposure, predator avoidance performance of larval fathead minnows was assessed using a high-speed camera set at 1000 frames/second. By integrating biological results gathered across a range of species and encompassing varying degrees of experimental controls we are able to examine the robustness of screening tools for endocrine disrupting compounds in an environmental setting.

James C. Lamb | *Exponent*

9 Critical Comments: WHO and UNEP State of the Science of Endocrine Disrupting Chemicals 2012

P. Boffetta, Icahn School of Medicine at Mt Sinai; W. Foster, McMaster University; J. Goodman, Gradient; K. Hentz, Exponent; L. Rhomberg, Gradient; J. Staveley, G. Swaen, Exponent; G. Van Der Kraak, University of Guelph; A. Williams, Exponent

In 2002, the World Health Organization (WHO) and International Programme on Chemical Safety (IPCS) published a "state-of-the-science" assessment of endocrine disruptors summarizing the prevailing state of scientific knowledge, including data gaps and prospects for resolving uncertainties with further research. In 2012, the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) "updated" the 2002 report. We identify several significant concerns that raise questions about conclusions in the 2012 report regarding the strength of evidence for endocrine disruption. First, the report is not a state-of-the-science review because it lacks a defined scope, a systematic approach for data collection/review, and a clear methodology for integration/assessment of data. Importantly, the report does not follow the 2002 WHO and IPCS weight-of-evidence approach and relies on "best professional judgment" to assess data. The basis for literature selection is not described, nor is study quality considered. Second, three factors are needed to show that a chemical is an endocrine disruptor: an endocrine-mediated mechanism of action, an adverse effect, and that these are causally related. Often only one or two of these factors are provided as evidence, so endocrine disruption cannot be established. Dose and potency of particular chemicals are also ignored. Third, a series of unrelated facts is often presented to infer that these facts are related. This leads to false impressions and conclusions that are not supported by the data presented. In particular, trends in disease incidence/prevalence are discussed without regard to their causes with the implication that they are explained by endocrine disruption. Finally, controversial topics are poorly addressed (i.e., low dose effects, non-monotonic dose response), and yet these are central to understanding potential effects of endocrine disruption. Overall, the 2012 report does not provide a balanced assessment or accurately reflect the state of the science.

James Wheeler | *Syngenta Ltd*

10 Guidance on Test Concentration Setting for Fish In Vivo Endocrine Screening Assays

G. Panter, AstraZeneca; L. Weltje, BASF; K. Thorpe, University of Portsmouth

Fish in vivo screening methods to detect endocrine active substances, specifically interacting with the hypothalamic-pituitary-gonadal axis, have been developed by both the Organization for Economic Co-operation and Development (OECD) and USEPA. In application of these methods, i.e. regulatory testing, this poster provides guidance on the setting of test concentrations using all available acute and chronic data and also discusses the importance of avoiding the confounding effects of systemic toxicity on endocrine endpoints. This guidance is aimed at reducing the number of false positives and subsequently the number of inappropriate definitive vertebrate studies potentially triggered by effects consequent to systemic rather than endocrine toxicity.

James Wheeler | *Syngenta Ltd*

11 Mind the Gap: Concerns Using Endpoints from Endocrine Screening Assays in Risk Assessment

L. Weltje, BASF; R. Green, Syngenta Ltd

Endocrine screening assays not only provide mechanistic information on the potential of a substance to interact with the endocrine system, but also data potentially relevant for risk assessment. However, these screening assays have a number of limitations that should be

considered before the direct use of such data for risk assessment purposes. This poster presents a proposal to provide an objective and transparent process in order to consider which endpoint(s) might be incorporated into a risk assessment, and when more definitive studies may be of value. Such an approach is necessary to ensure the appropriate use of screening data to further our understanding of the (eco) toxicological profile of substances undergoing screening.

Latonya Jackson | *University of Louisiana at Lafayette*

12 Lifetime Exposure of the Least Killifish (*Heterandria formosa*) to 17α-Ethinylestradiol: Organism- and Population-Level Effects

P. Klerks, University of Louisiana at Lafayette

The presence of endocrine disrupting compounds (EDCs) in the environment has gained worldwide attention because of the possibility that EDC's may be affecting the reproductive health of wildlife and humans. The synthetic estrogen 17α-ethinylestradiol (EE2) is a potent endocrine modulator and is present at biologically active concentrations in aquatic ecosystems. To investigate impacts of EE2 in the aquatic environment, a full life cycle in vivo study was done to examine the potential effects of EE2 in the least killifish (*Heterandria formosa*) at both the individual and population levels. I tested the effects of an environmentally relevant concentration of waterborne EE2. Newborn fishes were exposed to 5 ng/L EE2 or a solvent control (ethanol) in a static-renewal system with replacement every 72 hours. Effects of EE2 on length, weight, sex ratio, survival rate, and time to sexual maturity in the developing fishes were examined at the individual level. At the population level, population growth rates and changes in population were examined. My preliminary studies indicated that at 5 ng/L, EE2 caused a significant reduction in growth of females, female-biased sex ratio, decreased survival, and longer times to sexual maturity. There was also a trend of EE2-exposed males exhibiting faster growth than control males. At four months, there were notably fewer individuals in the exposed populations compared to the control populations. The control populations grew at a rate of 1.6% per month while the exposed populations exhibited no growth at all. Results for population growth rates and individual measurements for an entire breeding season will be displayed at the meeting. This ongoing study adds to the growing body of evidence of significant risks to aquatic organisms from exposure to environmentally relevant concentrations of EE2.

Laurent Lagadic | *INRA*

13 Development of In Silico, In Vitro and In Vivo Assays for the Evaluation of Endocrine Disruption in the Freshwater Gastropod *Lymnaea stagnalis*, Under the OECD Test Guideline Development Programme

M.A. Coutellec, V. Ducrot, INRA, Research Group on Ecotoxicology and Quality of Aquatic Environments, UMR0985 Ecology and Ecosystem Health

Lymnaea stagnalis has been selected as a test species for the development of an OECD Guideline for reprotoxicity testing in molluscs. This in vivo test is currently under validation and appears as a Mollusc Partial Life Cycle Assay at Level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (EDs). It is meant at providing data on adverse effects on endocrine relevant endpoints related to reproduction. For such an apical test, endpoints can be sensitive to more than one mechanism and measured effects may be due to non-ED mechanisms. Therefore, screening methods are needed to establish unequivocally endocrine disruption in *L. stagnalis*. Current research programmes of the INRA Research Group Ecotoxicology and Quality of Aquatic Environments aim at developing molecular-based screening methods for endocrine disruption in this freshwater gastropod. Transcriptomic and proteomic approaches have been implemented in order to identify response patterns specific of snail exposure to selected endocrine active compounds (EACs) (tributyltin, vinclozolin, chlordecone, pyriproxyfen). In parallel, these chemicals have been tested using Yeast Estrogenic and Androgenic Screening (YES and YAS) assays to confirm endocrine activity, and they have also been tested for their potential adverse reproductive effects in intact snails. Cross-comparisons between the results of YES and YAS assays, transcriptome and proteome response pattern analyses and in vivo reprotoxicity tests will allow assessing (i) the validity of non-specific screening methods for identifying endocrine disruption in *L. stagnalis* and (ii) the ability of omics to detect ED-mediated alterations of snail reproduction. In complementary experiments, the properties and ligand-affinity to EAC-binding receptors of *L. stagnalis* will be characterized. We will take benefit from the genomic resources recently acquired in this species to sequence *L. stagnalis* ER and RXR genes (LymER and LymRXR, respectively), to synthesize expression vectors of these genes, and to test their inducibility by ligands known to bind to human ER and RXR, using transient and stable transfection. This in vitro approach will contribute to fill the knowledge gap on ED-binding receptors in molluscs and strengthen interpretation of mechanisms underlying adverse reproductive effects of chemicals in *L. stagnalis* in the context of the forthcoming OECD Guideline. Finally, the ligand binding domains of LymER and LymRXR will be modelled in order to virtually screen a larger number of ligands for their specific endocrine activity in *L. stagnalis*. These in silico models will be used by the EDMON (Endocrine Disruptor MONitoring) software to model interactions of LymER and LymRXR with any type of contaminant. Overall, in silico prediction methods and in vitro assays which could be respectively implemented as levels 1 and 2 tests in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters will nicely support interpretation of partial and full life-cycle tests foreseen as levels 4 and 5 tests, respectively, within this framework.

Meredith Gooding Lassiter | *USEPA*

14 Use of a Cross-Species Approach to Evaluate Endocrine Disrupting Effects for the Integrated Science Assessment for Pb in Support of USEPA National Ambient Air Quality Standards

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The USEPA regulates six criteria air pollutants including lead (Pb) as mandated under the Clean Air Act, setting the National Ambient Air Quality Standards (NAAQS). Primary NAAQS provide public health protection, including protecting the health of at risk populations such as asthmatics, children, and the elderly. Secondary standards provide public welfare protection, including protection against decreased visibility and damage to animals, crops, vegetation, and buildings. Prior to setting the standards, the agency collects the most current and relevant science into the Integrated Science Assessment (ISA) to support decision making for the NAAQS. The ISA is a synthesis and evaluation of the most policy-relevant science that forms the scientific foundation for the review of the primary and secondary NAAQS. In support of the Pb NAAQS we conducted a

cross-species evaluation for primary and secondary standards which provides a unique opportunity to compare exposures to a common toxicant in the endocrine systems of laboratory animals and wildlife and to use these data in support of hazard identification within the NAAQS setting process. Both humans and non-human biota share pathways of transport, fate and exposure to Pb. In the 2013 ISA on Pb studies were reviewed that support Pb as an endocrine disrupting compound with impairments in maturation, fecundity, effects on sperm and sexual development or puberty onset that spanned multiple phyla, including effects on frog metamorphosis, a Tier 1 test, and cross-generational effects in nematodes. Pb-dependent disruption of estrous cyclicity and GnRH pulsatility, as well as suppression of serum LH, IGF-1, inhibin B, and estradiol give further support to Pb as an endocrine disrupting compound. Pb is also known to delay the onset of puberty as measured by multiple reports including Tanner staging in humans and vaginal opening timing in laboratory animals. Delayed maturation with Pb-exposure was noted in multiple species including worms, flies, insects, and frogs. These commonalities in responses to Pb are based on comparative biology and point to potential underlying common modes of action. The Pb ISA serves as an example of how studies from both toxicological and ecological disciplines can be used in a weight of evidence approach to report endocrine disrupting effects across species. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the USEPA.

Patricia L. Bishop | *People for the Ethical Treatment of Animals*

15 The Importance of Using Other Scientifically Relevant Information in the USEPA Endocrine Disruptor Screening Program to Reduce Animal Use and Avoid Duplicative Testing

C.E. Willett, The Humane Society of the US

The USEPA Endocrine Disruptor Screening Program (EDSP) currently uses an initial Tier 1 screening battery consisting of five in vitro and six in vivo assays to evaluate a chemical's potential to interact with the endocrine systems in mammals, fish and amphibians. Congress, in creating the EDSP, and the Office of Management and Budget, in approving the Tier 1 Information Collection Request, directed USEPA to also consider Other Scientifically Relevant Information (OSRI) that is directly or functionally equivalent to data gathered in Tier 1, in lieu of developing new test data. This study characterizes the types of OSRI submitted by chemical companies for the first 67 chemicals subject to EDSP test orders (List 1), how often USEPA accepted OSRI to grant waivers, and the distribution of those waivers among Tier 1 assays. Respondents for 47 of the List 1 chemicals submitted OSRI in hopes of satisfying some or all Tier 1 test requirements, with a combined total of 412 waivers sought. USEPA granted only 93 waivers, an overall acceptance rate of 23%. For 20 of the 47 chemicals, USEPA denied all OSRI and required the entire Tier 1 battery be performed. In most cases, the OSRI accepted either used a study method that was identical to the Tier 1 assay protocol or indicated a positive response by the chemical in question. Although identified as potential sources of OSRI in USEPA's guidance to test order recipients, Part 158 guideline studies for pesticide registration, such as the mammalian two-generation reproductive toxicity study and 90-day chronic rodent or dog studies, were seldom accepted by USEPA to satisfy Tier 1 data requirements. We show that USEPA treated OSRI inconsistently in some cases and often applied a check-box approach, rather than a weight-of-evidence approach, to evaluating the existing data. While the 93 waivers reduced animal use by at least 3,325 animals, this is a small reduction considering that 27,731 animals were used to perform Tier 1 tests on the List 1 chemicals. Additional animals were used by labs when preparing to run the assays. Even with USEPA's plans to shift toward applying 21st Century toxicology tools to screening of endocrine disruptors in the future, acceptance of OSRI will remain a primary means for avoiding duplicative testing and reducing use of animals in the EDSP. Therefore, it is essential that USEPA develop a consistent and transparent basis for accepting OSRI.

Paula F. P. Henry | *USGS Patuxent Wildlife Research Center*

16 Hormone Biochemical Measures for Monitoring Avian Response to Endocrine Disruptive Compounds

N.K. Karouna-Renier, USGS Patuxent Wildlife Research Center

The USGS Patuxent Wildlife Research Center is interested in developing rigorous methods for evaluating potential effects of EDC on wild avian species in the field. Hormone concentrations are one of the critical endpoints of the endocrine system. As such, ratios of hormone levels in exposed relative to control groups are used as biochemical measures for endocrine disruption. The USEPA and OECD avian two-generation tests are designed for the Japanese quail model. For the F0, F1 and F2 generations, analyses of specific plasma, fecal, or urate hormones are selected based on the presumptive endocrine activities of the contaminant. Correlating effects such as survival, embryonic development, egg production, or reproductive success, to alterations in hormone concentrations by nature, involves considering multiple feedback pathways. Additionally, variations in relative sensitivity across species can be attributed to differences in receptor binding capacity, gene and enzyme products, or the robustness of specific markers. How does the response of the Japanese quail model relate to that observed in a bird found at a contaminated site in the field? We investigate and discuss applying hormones as endpoints in field birds relative to hormonal findings from tests conducted with the Japanese quail.

Richard A. Becker | *American Chemistry Council*

17 Interpreting Estrogen Screening Assays in the Context of Potency and Human Exposure Relative to Natural Exposures to Phytoestrogens

S.M. Hays, C.R. Kirman, L.L. Aylward, Summit Toxicology; K. Wise, American Chemistry Council

Indication of exposure to potentially endocrine active substances can be detected in urinary biomonitoring studies in the general population. While USEPA and OECD have developed and validated in vitro and in vivo screening assays to measure interaction of substances with estrogen, androgen and thyroid pathway components, to date, methods to contextualize such results in terms of potencies and actual human exposures are lacking. Although urinary concentrations of substances can be compared on a mass per volume basis, such comparisons are not biologically meaningful because a receptor response is dependent upon potency and the concentration of the active moiety at the receptor. To understand endocrine screening results in terms of potency and human exposure, we propose a method that entails 1) calculating a benchmark dose (BMD) for a response measured in an endocrine screen; 2) estimating the human urinary concentration (Biomonitoring Equivalent, BE) expected to correspond to this dose (BEBMD); 3) deriving the Exposure:Activity Ratio (EAR) by comparing actual urinary values from human biomonitoring studies (e.g. NHANES) to the BEBMD; and 4) comparing the EAR for a substance to one for a reference chemical. Using OECD uterotrophic assay validation studies and NHANES results, we calculated EARs

for genistein (EARGEN =6.6E-4), and bisphenol A (EARBPA =8.8E-7). GEN, a phytoestrogen in a healthy diet, is recommended as a reference to interpret the significance of an EAR for estrogenic response, and to calculate the Relative Endocrine Exposure Activity Quotient (REEAQ), defined as EARCMPND ÷ EARGEN. The REEAQBPA/GEN =1.34E-3, indicates the EAR (uterotrophic) for the geometric mean level of GEN exposure is more than 700-fold greater than the EAR (uterotrophic) for typical BPA exposures in the US population. These methods may be applied to additional compounds and endocrine assays and can assist in decisions regarding the need for additional endocrine screening and testing; substances with low EARs and low REEAQs may not warrant additional testing.

Simon Dinehart | *ABC Laboratories, Inc.*

18 Performance of and Challenges Encountered during Larval Amphibian Growth and Development Assays (LAGDA) Using *Xenopus laevis* Following the OECD Draft LAGDA Guideline

S. Hicks, ABC Laboratories, Inc.; R. Kropp, Battelle; S. Lynn, USEPA

The testing and assessment of chemicals for endocrine disruption properties has been a priority of the USEPA and regulatory authorities in Organisation for Economic Co-operation and Development (OECD) member countries. The USEPA has adopted a two-tiered approach as part of the Endocrine Disruptor Screening Program (EDSP), with Tier 1 tests focusing on screening chemicals for potential endocrine activity and Tier 2 tests for confirmation and characterization of endocrine activity identified in Tier 1. An important step in EDSP implementation is the evaluation and validation of ecotoxicological Tier 2 tests that will serve as a basis for the risk assessment of chemicals and environmental contaminants with endocrine activity. The LAGDA is designed to evaluate overt-toxicity and effects on endocrine-mediated physiological processes (e.g., gonadal differentiation) which are conserved across vertebrate species. ABC Laboratories evaluated survival, development, growth, and secondary biomarker expression (vitellogenin and T4) in *Xenopus laevis* following exposure to 4-tert-octylphenol and prochloraz (two chemicals with well characterized modes of action as reported in peer review literature) from embryonic to juvenile developmental stage following the draft OECD LAGDA guideline. Endpoints were measured prior to and at the completion of metamorphosis [Nieuwkoop-Faber (NF) stage 62 and NF stage 66 animals, respectively], and in post-metamorphic juveniles (10 weeks post-metamorphosis). With several exceptions, both tests satisfied the validity and performance criteria stated in the draft OECD LAGDA guideline. The challenges encountered during study conduct (e.g., obtaining sufficient viable embryos for test initiation, achieving and maintaining acceptable exposure concentrations) and the implemented solutions will be detailed. This abstract does not necessarily reflect USEPA policy.

Kimberly J. Wooten | *Texas Tech University*

19 In Vitro Androgenic and Estrogenic Activity of Particulate Matter Emitted from Cattle Production Facilities

B.R. Blackwell, A.D. McEachran, P.N. Smith, Dept of Environmental Toxicology, Texas Tech University

Cattle production facilities, including feedlots and dairies, contain large numbers of cattle in relatively small spaces and thus generate significant amounts of organic waste. Many of these facilities are open-air, meaning waste has the potential to enter the environment as airborne particulate matter (PM), especially in the arid conditions that exist where many beef cattle feedlots are located. Recent research has confirmed that PM emitted from feedlots includes quantifiable amounts of both natural and synthetic steroids (i.e. 17α- and β-trenbolone, α- and β estradiol); there is limited data, however, on the potential effects of these steroids after they enter the environment via aerial transport. The goal of the current study was to determine if feedlot- and dairy-emitted PM elicits androgenic and/or estrogenic activity in vitro using transcriptional activation assays. Particulate matter samples were collected from upwind and downwind sides of feedlots (n=5) and dairies (n=5). PM mass collected was larger downwind than upwind of feedlots ($p<0.0001$), but not dairies ($p=0.14$). Feedlot collected PM elicited both androgenic and estrogenic activity in vitro, with downwind samples having greater androgenic ($p<0.0001$) but not estrogenic ($p=0.33$) activity. Dairy samples did not elicit estrogenic activity in either upwind or downwind samples. Results from the screening level in vitro assays used in this study suggest that PM emitted from feedlots in particular may warrant further investigation to determine its potential for endocrine disruption in organisms in close proximity to feedlots.

Stephen L. Hicks | *ABC Laboratories, Inc.*

20 Evaluation of Proposed Ecotoxicological Testing Guidelines Using Mysids to Determine Endocrine Disruptive Effects

J. Aufderheide, ABC Laboratories, Inc.; R. Kropp, Battelle; R. Hall, L. Touart, USEPA

WITHDRAWN

The testing and assessment of chemicals for endocrine disruption has been a priority of the USEPA and regulatory authorities in Organization for Economic Co-operation and Development (OECD) member countries. The USEPA has adopted a two-tiered approach as part of the Endocrine Disruptor Screening Program, with Tier 1 tests focusing on screening chemicals for potential endocrine activity and Tier 2 for confirmation and characterization of specific endocrine activity identified in Tier 1. Tier 2 testing focuses on the partial or multi-generation-life-cycle of several animal groups. The proposed mysid two-generation test is an in vivo Tier 2 assay providing an invertebrate model for a sexually reproducing species that could be detrimentally affected by endocrine active substances. Study protocols were developed following the OECD Draft Proposal for a New Guideline; Mysid Two-Generation Test Guideline, drafted August 2006. Assays conducted at ABC Laboratories will be compared to recommended acceptance and performance criteria presented in the revised test guideline. The performance of control mysids during two assays utilizing the self-starting siphons and guideline-specified feeding regime did not meet all acceptability criteria. Specifically, average total of young produced per control F0-female mysid was less than nine and in one instance the number of control F0-female mysid producing young was less than 75%. Six additional assays were performed using a diet of enriched brine shrimp nauplii offered to mysids ad libitum and without self-starting siphons. Control mysids during these six assays did meet the test acceptability criteria. The challenges experienced during testing will be presented along with steps taken to overcome some of these challenges. Recommended revisions to the draft guideline were made at the conclusion of testing and some of these recommendations which have been incorporated into the most recent version of the draft OCSPP 890 and OECD Mysid Two-Generation Test New Test Guideline drafted July 2013 will be discussed. This abstract does not necessarily reflect USEPA policy.

Teri S. Myers | *CDM Smith*

21 A Sweet Consideration: How Could High-Sugar Diets Impact Endocrine Disruption Assays With Organophosphates?

T.M. Myers, Analytical Toxicology Division, Neurobehavioral Toxicology Branch, US Army Medical Research Institute of Chemical Defense (USAMRICD)

This paper will demonstrate the need to consider the effect of high-sugar diets in laboratory testing of endocrine-disrupting chemicals. A glucose-enriched diet has been shown to double the toxicity of organophosphates to rats, relative to a standard laboratory diet containing comparatively lower amounts of sugar. Additionally, organophosphates are known to disrupt endocrine systems in mammals. These known effects are particularly important because sugar consumption in humans is quite high. In fact, recent research has shown that human sugar consumption has risen 45% in the last 30 years and that the US is the country that ranks highest in per capita sugar consumption. Evaluating the impact of a high-sugar diet in laboratory mammals on endocrine system disruption by organophosphates may reveal different findings than the current assays conducted with standard laboratory chow. Consideration of endocrine-disrupting effects during consumption of high-sugar diets could improve translations of laboratory study results to real-world human implications.

AtLee T. D. Watson | *North Carolina State University*

22 Sublethal Exposure to TCDD Impacts Axial Osteogenesis in Japanese Medaka (*Oryzias latipes*)

S. Kullman, Dept of Biological Sciences, North Carolina State University

2,3,7,8-tetrachlorodibenzodioxin (TCDD, dioxin), a well-known endocrine disruptor, functions through activation of the aryl hydrocarbon receptor (AhR). Recent studies from mammalian, fish, and in vitro models have identified bone and cartilage development as sensitive target for dioxin and other AhR ligands. In this study, we assess how low-level embryonic TCDD exposure impacts axial osteogenesis in Japanese medaka (*Oryzias latipes*), an vertebrate fish model. Embryos from three transgenic medaka tg(twist:EGFP; osx:mCherry; col10a1:nlGFP) were exposed to a range of TCDD concentrations and reared to 10-20 dpf. Individuals were stained for mineralized bone matrix and imaged to assess skeletal alterations within rostral and medial vertebrae in relation to qualitative spatiotemporal analysis of osteoblast and osteoblast progenitor cell populations. TCDD exposures impacted axial osteogenesis through an overall attenuation of skeletal mineralization resulting in truncated centra, increased intervertebral ligament area, and reduced neural and hemal arch length. At later stages of development this effect was more pronounced. Effects on mineralization were consistent with modifications in cell number and cell localization of labeled osteoblast and osteoblast progenitor cells. Our results indicate that sublethal TCDD exposure impacts either proliferation and/or differentiation of osteoblasts during critical periods of bone growth and mineralization in medaka. Further work is underway to anchor phenotypic data with gene expression and extrapolate effects to human health using mammalian in vitro models.

Virginie Ducrot | *INRA*

23 Development of an OECD Standard Reproductive Toxicity Test with the Freshwater Gastropod *Lymnaea stagnalis*: Current Status and Further Steps

D. Azam, INRA; R. Brown, AstraZeneca, Brixham; S. Charles, University Claude Bernard; H. Holbech, University of Southern Denmark; T. Hutchinson, University of Plymouth; P. Matthiessen, Independent Consultant; Jörg Oehlmann, Goethe University; L. Weltje, BASF; L. Lagadic, INRA

The pond snail *Lymnaea stagnalis* has been selected as a test species for the development of an OECD guideline for reprotoxicity testing in molluscs. It aims at providing data on adverse effects of chemicals, including those with endocrine activity in vertebrates, on reproductive endpoints. As such, this in vivo test will appear as a Mollusc Partial Life Cycle Assay at Level 4 of the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. We present a summary of the protocols implemented for both snail mass culture and a reproduction test, as well as the results obtained in the pre-validation ring-test using the draft standard protocol. All the snails used for the ring-test came from the Renilys® strain, mass-reared at INRA Rennes. Animals were checked for homogenous size, shell integrity, ability to reproduce, and the absence of parasites. They were then dispatched to eight partner laboratories. Semi-static tests were performed after snail acclimation, with a range of nominal concentrations of cadmium chloride (53, 80, 120, 180, 270, 405 and 608 µg/L of Cd) and tributyltin chloride (75, 150, 300, 600 and 1200 ng/L of TBT). Cd concentrations measured after 15 minutes confirm correct application of the products in all laboratories. This was also the case for TBT concentrations, which could be correctly applied using glacial acetic acid (0.001% v/v) was used as a carrier solvent. Water quality validity criteria were met by all laboratories i.e. dissolved oxygen content > 60% ASV and temperature 20 ± 1°C. The biological validity criteria were also met, with control snail survival > 80% and threshold level for minimal egg mass production per snail over the test period (56 days) in controls > 8. Control reproductive output was reproducible among experiments conducted in a given laboratory. A maximum two-fold difference occurred when comparing the reproductive outputs in controls from the various laboratories, which did not hamper ECx estimations. All snails exposed to Cd at one of the participating laboratories died after 21 days, whatever the concentration, which hampered the assessment of reproductive effects in snails from this laboratory. The reason for this unexpected mortality was the softness of the test water (< 50 mg CaCO3/L) used in this laboratory. The draft protocol was amended to provide guidance on appropriate water hardness for toxicity tests with *L. stagnalis*. In all other laboratories, significant effects of Cd on reproduction were observed, allowing the derivation of concentration-response relationships. Comparable values for reproductive endpoints after 56 days were obtained (68 < EC50 < 124 µg/L; n = 6) and were consistent with published literature. Reprotoxic effects of TBT were observed and concentration-response relationships could be derived. Reproduction endpoints values after 56 days were not significantly different between laboratories (303 < EC50 < 452 µg/L; n = 3) and were also consistent with published data. Coefficients of variation showed for both compounds that EC50-56d values were fairly reproducible among laboratories (< 30%), while EC10-56d scattered more. Data analysis showed that test duration can be reduced to 38 days without modifying NOEC/LOEC and EC50 estimates. A validation ring-test using the revised protocol has started in October 2013 with 15 partner laboratories in Europe and North and South America. Cd and TBT are tested by 8 laboratories which were not involved in the pre-validation study, to assess the

reproducibility of results. New chemicals are also being tested, including prochloraz and trenbolone, in order to validate the protocol with a larger number of chemicals.

Allen Olmstead | *Bayer CropScience*

24 MAMA – Modified Amphibian Metamorphosis Assay – An Alternative Test Design

C. Banman, SynTech Research; L. Ortego, T. Hall, Bayer CropScience

The performance of the Amphibian Metamorphosis Assay (AMA) that is included in the USEPA Endocrine Screening Program (EDSP; Guideline number OCSPP 890.1100) has recently been evaluated by a Science Advisory Panel (SAP) convened by the USEPA. The current guideline is designed as a 21-day test. It is highly desired that the tadpoles do not reach NF stage 60 by day 21, which assumes some synchronicity in egg development. The SAP evaluated a subset of AMA testing results collected so far, and questions were raised as to how to account for differential tadpole growth rates, metamorphosis timing and development of the thyroid during the test since the animals will be at various stages of development when sampling occurs at fixed time points. These endpoints are not independent and will influence the interpretation of the study endpoints (e.g., snout-vent length, hind limb length, thyroid histology) which are taken at fixed times during the study (Day 7 and Day 21). When a test compound has the potential for an effect on amphibian metamorphosis, data analysis is confounded by the fact that numerous labs have had difficulty in the synchronicity of metamorphosis and obtaining an appropriate distribution of developmental stages. And when this occurs pre- and post-NF stage 60 frogs cannot be compared due to dramatic morphological remodeling that takes place during climax, nor is it appropriate to compare thyroid histology in developing organisms across non-stage matched tadpoles. An alternative test design has been developed that will control for these interactions. In the alternative design, experimental set-up, acclimation, initiation, and daily observations are similar to the current guideline. Day 7 endpoints are the same (hind limb length, snout vent length, wet body weight, and developmental stage). However post-day 7, tadpoles are observed daily and those having both forelimbs erupted (NF developmental stage 58) will be removed from the test tanks, endpoints measured (as noted on Day 7) and tadpoles processed for histological analysis. In this study sodium perchorate was used as the model thyroid active substance to enable comparison previous AMA studies using this compound. The new test design will allow appropriate comparisons of endpoints across the same developmental stage, and hence allow for a more robust analysis and interpretation of the study results.

Catherine A. Picut | *WIL Research, LLC*

25 Post-Natal Development of Testes in the Rat

D. Dixon, NIEHS; C. Swanson, L. Parker, A. Remick, WIL Research, LLC

There are stage-specific microscopic features of the developing testis in the rat from birth through puberty at post-natal day 55 (PND 55). Identifying these features assist the pathologist in interpreting the terminal histopathology in juvenile toxicity studies including the Male Pubertal and Thyroid Function Assay (PND 52/53). Testes from 168 Sprague-Dawley rats (3 animals per day from PND 0 through PND 55) were fixed in modified Davidson, sectioned at 5 µm, stained with H&E and PAS-H, and examined by light microscopy. During the neonatal period (PND 0 – PND 7), tubules were comprised of gonocytes and contained characteristic mitotically active immature Sertoli cells. During the early infantile period (PND 8-14), profound proliferation of spermatogonia into a pseudostratified layer occurred, while Sertoli cells continued to proliferate as their nuclei retreated to a basilar location. During the late infantile period (PND 15-20) the crowded spermatogonia reached maximum density forming a characteristic rosette. Leptotene/zygotene spermatocytes appeared centrally as a tubule lumen developed, and inter-tubular staging became apparent. The Juvenile period (PND 21-32) was characterized by dramatic increase in number and size of pachytene spermatocytes with formation of round spermatids at PND 26, and loss of "infantile" rosette architecture. The hallmark feature of the peri-pubertal period (PND 32-52) was spermiogenesis with stage VII tubules visible at PND 46. Knowing stage-specific features of testes during postnatal development helps the pathologist distinguish delayed development from other toxic effects, and determine pathogenesis when confronted with non-specific findings of hypospermatogenesis.

Catherine A. Picut | *WIL Research, LLC*

26 Post-Natal Development of the Ovary in the Rat

A. Remick, L. Parker, C. Swanson, D. Dixon, WIL Research, LLC

This study documents stage-specific microscopic features of the prepubertal ovary in the rat from post-natal day (PND) 0 through the juvenile period (PND 32). The microscopic features of the neonatal, infantile and juvenile ovary are correlated with published changes in LH/FSH levels, changes in the excitatory or inhibitory nature of GABA, development of the estrogen positive feedback loop, and adrenarche. Ovaries from Sprague Dawley rats (3 animals per day) were collected from PND 0 through PND 32, fixed in formalin, sectioned at 5 µm and stained with H&E. During the neonatal period (PND 0 – PND 7), primordial follicles were the predominant feature with densely packed 10 and 20 follicles in the core. During the early infantile period (PND 8-14), the predominant feature was "atypical" 20 follicles, often arranged in linear arrays reminiscent of ovigerous nests. The late infantile period (PND 15-20) was characterized by a predominance of "mature" 20 and early 30 follicles, which were evenly distributed throughout the parenchyma. Waves of follicular atresia depleting the medullary follicles characterized the juvenile period (PND 21-32). Knowing the stage-specific features of ovarian development and their correlation to the neuroendocrine profile allows the pathologist to better interpret morphologic changes in the ovary for the pubertal assay (PND 43) and other reproductive toxicity tests.

Donald G. Stump | *WIL Reseach, LLC*

27 A Comparative Assessment of Four In Vivo Assays Included in the EDSP Tier 1 Screening Battery in the Sprague Dawley Rat

P.S. Coder, E. Tanchevski, J.L. Mesnard, E.D. Sloter, WIL Reseach, LLC

In 2009, the USEPA published the EDSP Tier 1 battery of assays, study guidelines for conducting these assays, and the first list of around 65 chemicals with test orders. Between 2010 and 2012, WIL Research conducted over 60 studies under the EDSP. Here, we present a comparative assessment of control data derived from four of the rodent in vivo assays included in the Tier 1 Screening Battery – Uterotrophic, Hershberger and the male and female Pubertal assays in the Sprague Dawley rat. Parameters presented include adult male and female body, and reproductive organ weights, pup growth parameters, developmental landmarks (vaginal patency and balanopreputial separation), female estrous cyclicity, serum chemistry, serum hormones (T4, TSH, and Testosterone), organ weights, including accessory and primary reproductive organs and histopathological findings.

Interpretation of data obtained during the conduct of developmental and reproductive toxicity studies typically involves the widespread use of historical control datasets as a basis for interpretation. Due to the recent introduction of the pubertal assay guidelines and the infrequent use of the Uterotrophic and Hershberger assays prior to their inclusion in the Tier 1 Battery, relatively little historical control data was available for reference during these studies. This presentation is an effort to mine the extensive data obtained from our EDSP studies to identify discernible trends in pubertal development, endocrine physiology and alterations in reproductive organ weights (both adult and juvenile) following exposure to a variety of chemicals tested under the Endocrine Disruptor Screening Program.

Raanan A. Bloom | *US Food and Drug Administration*

28 Addressing Endocrine Disruption Potential of Drugs at the FDA Center for Drug Evaluation and Research

J. Laurenson, Center for Drug Evaluation and Research, US Food and Drug Administration

The US Food and Drug Administration (USFDA) Center for Drug Evaluation and Research (CDER) addresses the potential for a drug to disrupt the endocrine system as part of the pre-clinical toxicology review. A review of ecological effects following excretion and disposal of drugs into the environment is also conducted for certain drug applications. For human health effects, CDER recently announced the availability of a draft guidance for industry entitled, "Endocrine Disruption Potential of Drugs: Nonclinical Evaluation." This draft guidance provides recommendations to sponsors on the parameters that should be routinely assessed in toxicology studies for investigational new drug applications (INDs), new drug applications (NDAs), and biologics license applications (BLAs) to determine the potential for a drug to disrupt the endocrine system. This draft guidance also discusses factors to consider in determining the need for additional studies to characterize potential endocrine disruptor properties of a drug. Endocrine disruptors are defined in this guidance as drugs that produce an adverse response in an organism or its progeny by interfering with some aspect of the endocrine system. The draft guidance, when finalized, will represent the Agency's current thinking on nonclinical evaluation of endocrine disruption potential of drugs. For ecological effects, CDER is involved with a number of activities aimed at assessing the potential ecological effects from pharmaceuticals, including the potential for a drug to disrupt the endocrine system. These efforts include collaborations both internally with other USFDA Centers, and externally with other Federal agencies and groups. CDER also is prioritizing pharmaceuticals for risk assessments on environmental exposure and effects. The research and analysis conducted thus far has provided CDER with new information to consider going forward during drug application reviews. CDER is continuing to monitor new research to see whether the conclusions on which CDER's procedures for compliance with the National Environmental Policy Act (NEPA) are based have changed, and whether and how CDER's NEPA guidance should be modified.

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