Title: Endocrine disruption: where are we with hazard and risk assessment?

Running head: Hazard and risk assessment of endocrine disrupters

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ABSTRACT

Approaches to assessing endocrine disruptors (EDs) differ across the globe, with some regulatory environments using a hazard-based approach, while others employ risk-based analyses. In session four of the Society of Environmental Toxicology and Chemistry (SETAC) North America Focused Topic Meeting: Endocrine Disruption Chemical Testing: Risk Assessment Approaches and Implications (February 4 – 6, 2014), various aspects related to the hazard and/or risk assessment of EDs were explored. The presentations in the session included an overview of the regulatory environments for assessing and managing endocrine disruptors, and scenarios whereby a hazard-based approach might be most appropriate were discussed. Three case studies for ED assessment, one for an industrial chemical, one for a pharmaceutical, and one for a pesticide, were presented. The topics of non-monotonic dose response relationships as well as potency and threshold effects were also presented in this session, since these concepts are important for determining whether a risk or hazard based approach to ED regulation is most appropriate. Session four concluded with an open discussion concerning the issue of hazard and risk as a basis for regulating EDCs. An outcome of session four was the drafting of an outreach statement that summarizes the overarching themes of this session.

Keywords: Endocrine disruption, Hazard, Risk, Alkylphenols, Glyphosate, Ethinyl Estradiol

INTRODUCTION

In session four of the Society of Environmental Toxicology and Chemistry (SETAC) North America Focused Topic Meeting: Endocrine Disruption (February 4 – 6, 2014), various aspects related to the hazard and/or risk assessment of endocrine disruptor chemicals (EDCs) were explored. Peter Matthiessen presented an overview on the divergent approaches to managing EDCs in the United States and European Union. Holly Zahner and Jane Staveley presented background information and current regulatory initiatives for assessing EDCs in the United States, Japan, and Canada. Three case studies of endocrine evaluations were presented using 1) industrial chemicals, 2) a pesticide chemical, and 3) a pharmaceutical. In the industrial chemical case study, Katherine Coady discussed incorporating potency, critical effects, exposure, and risk assessment in the endocrine evaluation of the chemical intermediates, nonyl and octylphenol. The next presentation focused on a pharmaceutical example; Daniel Caldwell pointed to the value of effects-based measurements for EDCs rather than regulating on a chemical specific basis. In the case study for a pesticide compound, Steve Levine presented several lines of evidence that collectively indicate that glyphosate does not interact with the estrogen, androgen or steroidogenesis pathways, nor does it interact with the hypothalamus-pituitary-gonadal or hypothalamus-pituitary-thyroidal axes. Earl Gray presented findings on the occurrence of threshold, linear no threshold, and non-monotonic dose-responses from a survey of the toxicology literature, and overall concluded that while there were several instances of linear no threshold and non-monotonic dose responses, these occurrences did not influence the outcome of a risk assessment. In the final presentation of this session, Chris Borgert emphasized that the fundamental principles governing hormonal effects dictate the existence of thresholds for hormonal activity and also define the potential for exogenous chemicals to interfere with normal

endocrine functioning. Session four concluded with an open discussion concerning the issue of hazard and risk as a basis for regulating EDCs. An outcome of session four was the drafting of an outreach statement that summarizes the overarching themes of this session.

SESSION PRESENTATION SUMMARIES

Perspectives on Hazard- And Risk-Based Approaches to the Evaluation of Endocrine Disrupting Chemicals by: Peter Matthiessen

There is a divergence between how endocrine disrupting chemicals (EDCs) are to be regulated in the United States (US) as compared with the European Union (EU). Although the phenomenon of endocrine disruption was first recognized as such in the 1980s, it is only now that major jurisdictions such as the USA and EU are deciding how EDCs should be assessed and managed. A major reason for the delay has been the need to develop and internationally standardize a suite of new toxicity screens and tests that evaluate for potential adverse effects through an endocrine mechanism, a huge task which has made great progress, but is still under way at the Organization for Economic Cooperation and Development (OECD).

In the US, the Endocrine Disruptor Screening Program (EDSP) has begun deploying a Tier 1 battery of screens on chemicals to which humans and wildlife are widely exposed, and the intention is to conduct definitive testing at Tier 2 with those chemicals which, following a weight of evidence analysis of the Tier 1 data set (or equivalent data) along with other scientifically relevant information, show potential endocrine activity. Risk assessment and management will then proceed along traditional lines. In contrast, the EU has put legislation in place which will probably lead to most EDCs being prevented from entering the market, or being removed from

it, irrespective of whether humans or wildlife are exposed to toxicologically significant doses or concentrations. In other words, the EU proposes to regulate EDCs on the basis of their hazards and not their predicted risks. This process has not yet begun in the EU, however, because a regulatory definition of an EDC has still to be agreed upon.

The reasons for this divergence of approach are complex, but can be boiled down to a disagreement about the implications of various unique properties of EDCs for the safety of risk predictions. In summary, these properties include the following:

1. The ability of some EDCs to cause delayed but permanent damage to organisms after only short-term exposures during critical windows of development.
2. The concern that some EDCs are associated with non-monotonic dose-response relationships (NMDR), potentially making predictions of low-dose effects more difficult.
3. The alleged absence of toxic thresholds for some EDCs, which implies that there may be no safe levels of exposure.

In the US, and in many other jurisdictions, such as Japan, it is felt that these are not insuperable barriers to safe risk assessment. For example, some of the new toxicity tests are very sensitive to delayed toxic effects, and would also detect NMDRs (although the latter seem to be a phenomenon which rarely occurs with apical endpoints *in vivo*). The claimed absence of toxic thresholds also seems to be rare, if it occurs at all, and modern understanding of endocrine systems implies that they could not work without thresholds for agonistic action. Nevertheless, genuine scientific doubts about these issues have induced the EU to proceed with more caution than most other jurisdictions, with attendant implications for the continuing use, or appearance on the market, of many beneficial chemicals.

A SETAC Pellston workshop™ was proposed which would address these scientific questions through the evaluation of some comprehensive case studies. The of the workshop would be to identify scenarios in which risk assessment of EDCs is, and is not, a safe way to proceed. The intention was for the workshop to develop a guidance document which can be used by chemical companies and regulators when evaluating chemicals. In the meantime this workshop has been held and the output is currently under review for publication by IEAM[3].

Approaches to the Evaluation of Endocrine Disrupting Compounds at Several US and Foreign Government Agencies by: Holly M. Zahner and Jane Staveley

Many government agencies around the world are currently developing or implementing plans to evaluate the potential environmental impacts of endocrine disrupting compounds (EDCs), such as pesticides and pharmaceuticals. The approaches used to screen and test chemicals for their potential to interact with the endocrine system is dependent upon the legal authority of the government agency, which is why a fully harmonized approach both within the United States (US) and with other entities outside the US is not possible at this time. However, there is some overlap in the approaches used by some government agencies. The legal authority and approaches to screen and test for EDCs are described and compared for four government agencies (two in the US, one in Canada, and one in Japan).

The first and most well-known regulatory framework for screening and testing chemicals for their potential to disrupt the endocrine system is that of the US Environmental Protection Agency's (USEPA) Endocrine Disruptor Screening Program (EDSP; http://www.epa.gov/endo/). In 1996, the Federal Food Drug and Cosmetic Act (FFDCA) and Federal Insecticide Fungicide and Rodenticide Act (FIFRA) were amended with the Food Quality Protection Act (FQPA),

which mandated USEPA "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 the Administrator may designate." In addition, it required all pesticides (including both the active and inert ingredients) to be screened for endocrine disrupting activity. The EDSP was developed in response to this statutory mandate. Amendments to the Safe Drinking Water Act (SDWA) in 1996 also provided USEPA with authority to provide for testing of substances in drinking water sources, including EDCs (http://water.epa.gov/lawsregs/rulesregs/sdwa/index.cfm). The scope of authority given to USEPA under FQPA and SDWA covers approximately 10,000 chemicals. The first list of chemicals prioritized for testing under USEPA's EDSP (known as List 1) consisted of 67 pesticide active and inert ingredients, and the second list (known as List 2) consisted of 109 pesticide active ingredients and chemicals found in drinking water. The EDSP uses a two-tier screening and testing process. Tier 1 tests are used to identify chemicals that may have the potential to interact with the endocrine system, while Tier 2 tests are used to determine dose-related effects information on endpoints that are useful for risk assessments and can also be responsive and sensitive to endocrine modes of action.

There are other laws in the US that require the USEPA to evaluate the potential impacts of chemicals in the environment but do not have a specific focus on EDCs, including the Toxic Substances Control Act (TSCA) and the Clean Water Act (CWA). Under TSCA, USEPA has the authority to regulate all chemicals in commerce, with the exception of pesticides, foods, drugs and cosmetics, which are regulated under other authorities. There is currently an effort underway to modernize this statute, which was originally passed in 1976. The CWA focuses on surface water quality from both a human and ecological perspective by regulating discharges of pollutants to surface waters and setting standards for surface water quality. Consideration has

155 been given in recent years to developing aquatic life criteria for emerging contaminants detected
156 in surface waters (e.g., pharmaceuticals and personal care products). USEPA published a white
157 paper discussing the challenges of, and recommendations for, developing criteria for
158 contaminants of emerging concern, such as EDCs. USEPA used ethinyl estradiol (EE2), a
159 human pharmaceutical and potent EDC, in this paper as a model compound to demonstrate a
160 potential approach to the development of criteria for an emerging contaminant (USEPA 2008).

161 Other government agencies are also developing frameworks to address the environmental
162 risk of EDCs based on their regulatory authorities, including the US Food and Drug
163 Administration (USFDA), federal agencies in Canada (Environment Canada, Health Canada, and
164 the Pest Management Regulatory Agency), and Japan's Ministry of the Environment. The
165 USFDA's Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine
166 (CVM) assess the potential for environmental impacts from the use of EDCs (e.g., steroid
167 hormones) in human and veterinary pharmaceuticals under the National Environmental Policy
168 Act (NEPA) of 1969. NEPA mandates that all federal agencies in the US must consider the
169 potential environmental impacts of their actions. One type of agency action at USFDA is the
170 approval of a new or supplemental drug application. USFDA does not have a screening program
171 similar to EDSP to determine whether a drug may potentially disrupt the endocrine system;
172 however, it is often clear from the compound class (e.g., steroid hormones), structure, proposed
173 use, and/or other available data (e.g., mammalian toxicity data) that it may be an EDC. To
174 address the potential environmental impacts of EDCs, USFDA CVM is requiring that applicants
175 submit an environmental assessment (EA) as part of the application for approval of a new animal
176 drug product when the product contains a steroid hormone(s) and is to be used in food-producing
177 animals. In the EA, risks are to be evaluated from the use of the drug by comparing predicted

environmental exposure concentrations to predicted effect levels. If the EA adequately demonstrates that significant environmental impacts are not expected from the use of the proposed drug product, then USFDA will prepare a regulatory document known as a finding of no significant impact (FONSI) that is needed for approval of the drug application. In addition, USFDA CDER has recently published a Draft Guidance for Industry for comment titled "Environmental Assessment: Questions and Answers Regarding Drugs with Estrogenic, Androgenic, or Thyroid Activity Guidance for Industry" (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM444658.pdf; published on April 29, 2015). This guidance addresses specific considerations for human drugs that have potential estrogenic, androgenic, or thyroid hormone pathway activity (E, A, or T activity) in environmental organisms.

In Canada, there are two acts that govern the evaluation of environmental effects for chemical substances: the Canadian Environmental Protection Act (CEPA) and the Pest Control Products Act (PCPA). CEPA provides a definition for a "hormone disrupting substance" (Section 43) and states that "the Ministers shall conduct research or studies relating to hormone disrupting substances…" (Section 44.4), but neither of these acts has specific testing requirements or guidance on how to address the environmental impacts of hormone disrupting substances. These requirements will likely be described in the regulations when they are written. However, in the meantime, some attempt is typically made by regulators to consider potential hormone disrupting effects of pesticides and pharmaceuticals and the evaluation is generally based upon 1) identifying structural alerts or analogs to compounds known to exert endocrine effects, 2) evaluating submitted data for mammals, birds and fish for indications of potential endocrine-related effects, and 3) modeling potential interactions with receptors of interest. This

approach is similar to that used by the USFDA. In 2012, the Office of the Auditor General of Canada received a petition from Ecojustice and the Canadian Environmental Law Association requesting information about federal research activities on the effects of hormone disrupting compounds and, more specifically, how Environment Canada and Health Canada intend to use the results of this research in risk assessment and management of hormone disrupting substances. A response was prepared jointly by Environment Canada and Health Canada, which contains additional information on the Canadian government's activities with EDCs, and can be viewed at: http://www.oag-bvg.gc.ca/internet/English/pet_340_e_37607.html.

In Japan, the Ministry of the Environment has developed the EXTEND2010 (EXtended Tasks on Endocrine Disruption) program to assess the environmental risk of EDCs. This program promotes research, development of test methods, monitoring of environmental concentrations, effects assessment of selected chemicals (to include testing if necessary, in a tiered process), and risk assessment/management. The EXTEND2010 framework focuses on identifying actions on the endocrine system and characterizing the adverse effects to organisms. "Chemicals that can be subjected to tests for endocrine disrupting effects" are selected based on results from national monitoring programs and a reliability evaluation of existing data obtained from the literature. Similar to EPA's EDSP, the EXTEND2010 framework (http://www.env.go.jp/en/chemi/ed.html) has two tiers for assessing the effects of EDCs. Tier 1 consists of *in vitro* assays (reporter gene assays) and short-term *in vivo* assays using established test methods (e.g., fish short-term reproduction test, OECD guideline 229). Tier 1 considers all existing knowledge from the literature and test results to determine whether the compound may affect the endocrine system and whether additional analysis is required under Tier 2. Under Tier 2, a suite of *in vivo* chronic testing is recommended in invertebrates, fish, and amphibians to

characterize the endocrine disrupting effects of the compound of interest, including tests following OECD guidelines 230 and 231. Finally, an ecological risk assessment is conducted based on all of the available information in the literature and obtained from test results.

Octylphenol and Nonylphenol as Case Studies for Determining the Relevance of the Endocrine Mode of Action in Environmental Assessments by: Katherine Coady

Nonylphenol (NP) and 4-*tert*-octylphenol (OP) are chemical intermediates that are used in the manufacture of nonionic surfactants, phenolic resins, lacquers, antioxidants, and lubricating oil additives (Van Miller and Staples, 2005; Soares et al., 2008.) Most NP (65%) and a smaller fraction of OP are used to make the nonionic surfactants, nonylphenol ethoxylate (NPE) and octylphenol ethoxylate (OPE), respectively (Van Miller and Staples, 2005; Talmage, 1994; Soares et al., 2008). NPEs and OPEs are used in a wide range of products as emulsifiers, stabilizers, wetting agents, dispersants, and detergents (Talmage, 1994; Staples *et al.,* 2004; Soares *et al.,* 2008). NP and OP reach the aquatic environment primarily as degradation intermediates of NPE and OPE through wastewater treatment processes (Klecka et al, 2007, Melcer et al, 2007). NP and OP are slower to degrade and more toxic than their ethoxylates, and both NP and OP show a weak binding affinity for the nuclear estrogen receptor (Talmage, 1994; Servos, 1999; Environment Canada and Health Canada, 2001; Staples *et al*., 2004; Coady *et al*., 2010; Van Miller and Staples, 2005; Recchia *et al*., 2004; Olsen *et al*., 2005; Preuss *et al*., 2006; Van den Belt *et al*., 2004; USEPA, 2009). The estrogenic activity of NP and OP varies and is generally in the range of 1,000 - 1,000,000 fold less potent than the endogenous estrogen, 17β-estradiol (E2) (Coady et al., 2010; Van Miller and Staples, 2005; Wenzel et al., 2001).

While NP and OP have weak estrogenic activity, the adverse apical effects observed in fish exposed to NP and OP are not clearly endocrine mediated. In mixture studies with other estrogenically active compounds and NP and OP, the phenomenon of decreased fish reproduction due to OP exposure alone was clearly not solely attributed to estrogen-like activity (Brian et al., 2007). This mixture study concluded that OP "…exerts its effects on reproduction via more than one mechanism. The response pattern could be explained by a general toxic response…" (Brian et al., 2007). Furthermore, investigations using gene array technologies to specifically compare NP and E2 gene transcription profiles have established that NP has additional modes of action that are independent of the estrogen receptor (Larkin et al., 2002; Ruggeri et al., 2008; Watanabe et al., 2004). Molecular evidence in both mammalian and fish models have demonstrated that OP and NP influence a greater suite of genes than estrogens. For example, 425 genes were differentially expressed in liver tissue from zebrafish exposed to 10^{-7}M NP, while 153 genes were differentially expressed in liver tissue from zebrafish exposed to 10^{-7}M E2. Of the 30 most differentiated genes affected by NP compared to controls, only 1/3 of these genes were also altered among E2-exposed fish, and then not all in the same direction of change (Ruggeri et al., 2008). In mice, NP activated more genes than E2 in liver tissue, and the activated genes in the livers of NP-exposed mice were distinct from estrogen-responsive genes (Watanabe et al., 2004). These molecular studies of gene activation illustrate that NP and OP have multiple modes of action, of which weak estrogenic activity is one.

In chronic fish studies, NP and OP affect reproductive endpoints, such as sex ratio and spawning activity, at similar concentrations that affect growth and survival. Effects on growth and survival, as pointed out by the OECD guidance document on the assessment of chemicals for endocrine disruption, do not necessarily lead to a conclusion of endocrine disruption in fish

(OECD, 2011). Thus, the endocrine activities of NP and OP via binding to the estrogen receptor are not clearly the Critical effect[1] responsible for observed adverse effects in fish. In fact, the European Commission risk assessment on NP states: "Concentrations of nonylphenol at which oestrogenic effects are observed appear to be higher than those producing other effects" (European Commission, 2002). As an example, NOEC values in fish for OP based on reproduction range from 12 to 1,000 µg/L, while NOEC values based on growth range from 12 to 900 µg/L, and NOEC values based on survival range from 10 to 300 µg/L. Also, the most sensitive apical endpoints among fish toxicity studies with both NP and OP are based on decreased growth and survival (particularly in early life stage fishes), and not on endpoints that would be conceivably linked to the weak estrogenic activity of NP (Van Miller and Staples, 2005). Collectively, the NOEC levels for OP and NP for reproduction, growth and survival endpoints in fish all occur at very similar levels (Staples et al., 2004; Van Miller and Staples, 2005), indicating that the known weak estrogenic activity of NP and OP is not the sole, nor necessarily, the most sensitive, mode of action associated with observed adverse effects.

This signature of adverse effects on survival, growth, and reproduction occurring at similar concentrations is not the case when examining the toxic effects on fish exposed to potent estrogens. Estrogens affect sexual development and reproduction at concentrations that are far lower than the concentrations that cause acute lethality via narcosis, or baseline toxicity. For example, the 96-hr LC50 for zebrafish exposed to the synthetic estrogen, ethinylestradiol (EE2) was determined to be 1700 µg/L, and the NOEC for fertilization success (a reproductive endpoint) was 0.0003 µg/L EE2 in a lifecycle study with the zebrafish (Wenzel et al., 2001).

[1] Defined by EPA-IRIS as the first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases.

290 The ratio of these two endpoints is 5.73 x10^6 for EE2. In the same study design, the 96-hour
291 LC50 for zebrafish exposed to OP was determined to be 370 µg/L, while the NOEC based on
292 fertilization success was 12 µg/L OP (Wenzel et al., 2001). The ratio of these two endpoints for
293 OP is 31, and similar acute to chronic ratios can be calculated for NP. The relatively small acute
294 to chronic ratios for NP and OP are far different than the ratio of over a million that was evident
295 for EE2. These smaller acute to chronic ratios for NP and OP are more indicative of a narcosis
296 mode of action rather than a very specific and potent estrogen receptor binding mode of action.

297 Concentrations of NP and OP detected in the environment are below levels of concern for
298 environmental organisms. As part of the Water Framework Directive, surface water
299 concentrations of OP, NP, and numerous compounds have been measured in various European
300 waterways between 2007 and 2009 (DG Environment, 2009a; DG Environment, 2009b). From
301 this investigation, the median and upper 90th percentile concentrations for OP in surface
302 freshwaters in Europe was reported to be 0.05 and 0.25 µg/L, respectively, and the median and
303 maximum concentrations of NP in European surface waters were reported to be 0.03 and 0.460
304 µg/L, respectively (DG Environment, 2009a; DG Environment, 2009b). In North America, a
305 comprehensive review of the exposure data for NP and OP in surface waters revealed that the
306 average and upper 90th percentile concentrations for NP were 1.71 and 2.5 µg/L, respectively
307 (Klecka et al., 2007). OP concentrations were considerably lower in North America, with
308 average concentrations of 0.46 µg/L, and the complete range of reported concentrations of OP
309 spanning from 0.0003 to 1.10 µg/L (Klecka et al., 2007). In this review, it was noted that the
310 highest concentrations of OP and NP detected in surface waters were associated with effluent
311 dominated streams (Klecka et al., 2007). These NP and OP concentrations in both the U.S. and

312 European waters are generally well below NOEC and LOEC values from short term,
313 reproductive, and life cycle studies with NP and OP in aquatic organisms.

314 While both NP and OP do show weak estrogenic activity both *in vitro* and *in vivo*, it is
315 evident that they do not possess similar potency nor exert toxicity in the same pattern as natural
316 and synthetic estrogens. A close examination of both molecular data and data from chronic,
317 multigenerational studies with fish indicate that there are multiple modes of action of NP and OP
318 co-occurring within the same dose range. Regardless of the mode of action by which toxic
319 effects occur, concentrations of NP and OP in the environment are, by in large, too low to
320 adversely affect fish populations. These case studies with NP and OP illustrate the need to
321 incorporate the concepts of potency, critical effect, exposure, and risk in decision-making
322 regarding determinations of endocrine disruption and assessments of human health and
323 environmental impacts.

324

325 ***Magnifying Perceived Risk: A Case Study of Hazard and Risk Assessment of a***
326 ***Pharmaceutical Compound, 17α-Ethinylestradiol (EE2) by: Daniel J. Caldwell***[2]

327 Inaccurate or snapshot field measurements used as 'environmentally-relevant' test
328 concentrations in laboratory studies, biomarker detection (*i.e.*, vitellogenin in male fish)
329 incorrectly reported as an effect, and field experiments using confined exposure (*i.e.*, lake) being
330 inappropriately extrapolated to surface water (river) risk assessment have contributed to the
331 misconception that EE2 exposure is of great consequence to wildlife and humans.

[2] This talk was scheduled for the Society of Environmental Toxicology and Chemistry (SETAC) North America Focused Topic Meeting: Endocrine Disruption Chemical Testing: Risk Assessment Approaches and Implications, however was not able to be presented at that time. It is included here for completeness.

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 (Caldwell *et al.* 2012), and for humans potentially exposed via drinking water (Caldwell et al. 2010). However, continued attention is directed to this compound, including imposition of specific monitoring requirements in Europe. Monitoring or regulating individual substances ignores other estrogenic substances and will not eliminate responses in wildlife. A better approach is to establish a level of estrogenic activity that is without population impact and monitor waters for that endpoint. In this way, we identify 'hot spots' and can correct them, as the ultimate intent of the EU Water Framework Directive is to bring river basins to "good" ecological status.

There is evidence that EDCs with similar modes of action (MoA) can act together in an additive manner to produce effects. While some note that knowledge of MoA is necessary to be able to predict mixture toxicity, others indicate the more appropriate way is to base the prediction on common adverse outcomes (EFSA 2013; Report of the Endocrine Disrupters Expert Advisory Group 2013). There is a general agreement that the estimation of an experimental threshold in the case of mixed exposures is even more challenging and that information in relation to the MoAs (*e.g.* common or different MoAs of the ingredients of a mixture) is important for scientific understanding and for performing the appropriate risk assessment. In addition, there is not an adequate amount of scientific research to disregard other possibilities for combination effects of mixed exposures (*e.g.* synergistic, antagonistic action). For example, toxicokinetic and toxicodynamic interactions between chemicals may cause deviations on the shape of the dose response curves of individual chemicals (*e.g.*, inhibition of metabolism if substances are sharing the same metabolic pathway). Assessment of combination effects of chemicals in general, not

355 just EDCs, is already the subject of an initiative in the EU (Commission Communication to the
356 Council on the Combination Effects of Chemicals, 2012).

357 Proposals to implement compound specific environmental quality standards, such as
358 0.035 ng/L for EE2, will cost European countries billions of Euros to treat wastewater to remove
359 estrogens. For a UK town of around 250,000 people, such a system would cost €8 million to
360 install and €800,000 a year to operate - for the 1,400 facilities that would need upgrading in
361 England and Wales alone, this would amount to more than €30 billion in total (Owen and
362 Jobling, 2012). These costs will be borne by the public through higher water prices.

363 EE2, the estrogen ingredient in oral contraceptives, was estimated to be 1% of total
364 estrogen load excreted in the Dutch population in a paper that reviewed the literature regarding
365 various sources of estrogens in surface, source and drinking water and estimates that the risk of
366 exposure to synthetic estrogens in drinking water on human health is negligible (Wise et al.,
367 2011).

368 Monitoring data suggest that exposures of fish to EDC in surface water are largely due to
369 chemicals other than EE2 and that observed effects are likely due to the total estrogenic load, of
370 which EE2 is a minor contributor. A comprehensive assessment of EE2 exposure in Europe and
371 the United States, based on prescribed amounts of EE2, further supports this statement (Hannah
372 et al. 2009). This study by Hannah et al. used measured concentrations (MECs) taken from the
373 literature and predicted environmental concentrations (PECs) using the GREAT-ER and PhATE
374 models to develop expected exposure concentrations for surface waters of the US and EU. Key
375 findings were:

- 80% of all EE2 measurements globally show environmental concentrations below the detection limit of 0.1-1 ng/L and are consistent with modeled PECs.
- The highest MECs were not consistent with PECs, attributed to poor sample clean up or to inappropriate analytical methods.

The authors conclude that the 90th-percentile low-flow PECs of EE2 in surface water, conservative estimates of long-term exposure that should be used for risk assessment, are approximately 0.2 and 0.3 ng/L for the US and EU, respectively.

Thus, unless total estrogenic activity of surface water is addressed holistically we may miss important contributors to the total estrogenic exposure by focusing on individual EDCs rather than the mixture.

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, Bishenol-A, octylphenol, nonylphenol) that have demonstrated estrogenic activity.

Comparing the relative differences in occurrence and concentration with 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.

We reviewed measured concentrations of selected phthalates, bisphenol-A, octylphenol, nonylphenol, estradiol (E2), estrone (E1), estriol (E3), ethinyl estradiol (EE2), atrazine, and genistein in North America and Europe and compared them to aquatic predicted no effect concentrations (PNECs) (Caldwell et al 2009). Robust PNECs for the estrogens were derived by Caldwell et al. 2012. DEHP, BBP, and DBP PNECs were drawn from the Southern California Coastal Water Research Project Technical Report (Anderson et al., 2012), derived using the Ecosar chronic value / 100 or fish chronic NOEC / 100. PNECs for NP, OP, and BPA were bridged to E2 using VTG induction data presented in Brain et al. 2005, divided by 100. Genistein was bridged to E2 using the E-screen value of Falconer et al. 2006, divided by 100. A cumulative risk quotient (RQ) was calculated from the exposure concentrations and derived PNECs, with and without EE2 in the mixture. The RQ including EE2 was 124; without EE2 iT was 121.

Feminization in fish populations has been observed in a number of field surveys, but a detrimental impact on those populations has not been established nor been attributed to EE2 specifically. Based on the above RQ, it is unlikely that EE2 is a prominent contributor of the observed effects. Further, municipal wastewater effluents contain a variety of estrogenic compounds (including a significant component of female human origin) and EE2 is unlikely to play the prominent role in any estrogenic effects. The Dutch Ministry of the Environment concluded in 2010 that "in comparison with ethinyl estradiol, estradiol (and its transformation product estrone) is by far the greatest contributor to estrogenic activity in the aquatic environment."

417 Exposure to a mixture of EDCs has been predicted to result in additive effects, but this
418 has not been studied using environmentally relevant mixtures of EDCs. Yu et al. 2015
419 systematically investigated the estrogenic effects of 11 EDCs of high environmental concern
420 using the yeast estrogen screen (YES) method. The contribution of individual chemicals to the
421 total endocrine activity of environmentally relevant mixtures was evaluated using the ratio
422 previously determined (Caldwell et al 2009). On an individual basis, bisphenol-A, estrone,
423 estriol, ethinyl estradiol (EE2) and genistein showed estrogenic activity when compared with
424 estradiol, whereas bis(2-ethylhexyl) phthalate, octylphenol, nonylphenol, benzyl butyl phthalate,
425 and dibutyl pthalate showed anti-estrogenic activity. The full mixture of all these chemicals at an
426 environmentally relevant ratio also showed week anti-estrogenic activity. Further, EE2 did not
427 have a prominent contribution to the estrogenic activity of the mixture. The authors conclude
428 that a holistic evaluation of the estrogenic activity is necessary to evaluate the risk of a mixture
429 of endocrine active chemicals (EACs). This approach is also advocated in the EU by Kase and
430 colleagues (Kase et al. 2014), who recently introduced a project proposal for effect-based
431 monitoring approaches for steroidal estrogens under the EU Water Framework Directive.

432 EE2 is a minor contributor to the total estrogenic activity of surface water, yet is the topic
433 of much media coverage, which gives the public an inaccurate and incomplete risk profile.
434 Media emphasis on 'the pill' has misguided regulatory attention to focus on one component of an
435 endocrine active mixture. Unless estrogenic activity of surface water is addressed holistically
436 important contributors to the total estrogenic exposure may be missed by focusing on individual
437 EDCs. Rather than focusing on the detection of low levels of EE2, the effects of which are
438 known at true environmentally-relevant concentrations, efforts should go toward developing a

439 reliable estrogenicity assay to holistically determine the overall exposure that may result from
440 the mixture of EDC's that may be present. The Kase proposal has merit in this regard.

441

442 ***Regulatory Safety Studies and Tier 1 Endocrine Screening Assays Provide a Weight of***
443 ***Evidence that Glyphosate is Not an Endocrine Disruptor; Steven L. Levine***

444 Glyphosate (N-(phosphonomethyl)glycine, CAS number 1071-83-6) is a foliar non-
445 selective herbicide belonging to the phosphono amino acid class of pesticides. Glyphosate is a
446 specific inhibitor of one of the enzymes of the shikimate pathway, 5-enolpyruvyl-shikimate 3-
447 phosphate synthase (EPSPS), which is essential for the biosynthesis of aromatic amino acids and
448 other aromatic compounds in algae and higher plants, bacteria and fungi. Since the shikimate
449 pathway is found only in plants, bacteria and fungi, and not in animals, glyphosate generally
450 exhibits low toxicity to higher organisms, including mammals, birds, fish, aquatic invertebrates
451 and terrestrial invertebrates (Giesy et al. 2000).

452 In June 2007, EPA published in the Federal Register a notice announcing the draft list of
453 initial pesticide active ingredients and pesticide inerts to be considered for screening under the
454 Endocrine Disruptor Screening Program (EDSP). Chemicals were selected based on exposure by
455 three or four human exposure pathways that included food and drinking water consumption,
456 residential use exposure, and occupational exposure [70 FR 56449]. Throughout the selection
457 process, EPA clearly stated that "*this list should not be construed as a list of known or likely*
458 *endocrine disruptors. Nothing in the approach for generating the initial list provides a basis to*
459 *infer that by simply being on the list these chemical are suspected to interfere with the endocrine*
460 *systems of human or other species, and it would be inappropriate to do so*".

461 The Office of Management and Budget in its "Terms of Clearance" for List 1 compounds
462 stated that, "*EPA should promote and encourage test order recipients to submit OSRI in lieu of*
463 *performing all or some of the Tier I assays, and EPA should accept OSRI as sufficient to satisfy*
464 *the test orders to the greatest extent possible*" (OMB, 2009). Other Scientifically Relevant
465 Information (OSRI) is defined by EPA as "*information that informs the determination as to*
466 *whether the substance may have a similar effect produced by to a substance that interacts with*
467 *estrogen, androgen and thyroid systems*." In other words, information that informs the
468 determination refers to data of a suitable nature and quality that provides the same essential
469 predictive information even if different methods and procedures may have been used for
470 obtaining the data.

471 The Tier 1 EDSP screening battery tests whether there is the potential for endocrine
472 modulation through a specific endocrine mechanism(s) and not to assess if there is an adverse
473 effect through a non-endocrine mode of action. Tier 2 EDSP testing determines whether a
474 substance may cause endocrine-mediated effects through or involving estrogen, androgen, or
475 thyroid hormone systems, the potential consequences to the organism of the activities observed
476 in Tier 1, and establishing the relationship between dose and potential adverse effects for a
477 quatitative risk assessment. Therefore, results from Tier 1 and Tier 2 endocrine screening and
478 testing must be evaluated with a weight of evidence that includes a careful assessment of
479 potential overt toxicity. Consequently, dose setting for endocrine screening takes on great
480 significance to ensure that the interpretation of results are not confounded by overt toxicity and a
481 conclusion of hazard based on an endocrine mechanism is wrongly concluded (Marty et al.
482 2003). The analog for overt toxicity in *in vitro* assays are impacts to proteins in solution or
483 cytotoxicity to a cell line. Presently, the EDSP test guidelines permits $\leq$ 20% cytotoxicity before

a test concentration is eliminated from the analysis but no correction for cytotoxicity is considered. There are diagnostic tools for non-cell line *in vitro* assays to detect confounding effects that impact the stability of the assay environment such as denaturing or altering conformation receptors. Therefore, safeguards need to be in place to ensure that the assay is being conducted under proper biochemical conditions and there is proper data interpretation (Laws et al. 2007).

Prior to receiving Tier 1 test orders, the endocrine-modulating potential of glyphosate was rigorously evaluated in a variety of studies, including *in vitro* assays and standard *in vivo* toxicology studies capable of detecting adverse endocrine effects. Glyphosate *in vitro* assays demonstrate a lack of estrogenic, anti-estrogenic, androgenic and anti-androgenic activity and show no impact on steroidogenesis (Kojima et al. 2003; Petit et al. 1997; Hecker et al. 2011; Forgacs et al, 2012). Consistent with these *in vitro* findings, glyphosate was negative in the Tier 1 estrogen receptor (ER) and androgen receptor (AR) binding assays, the estrogen receptor transactivational activation assay, aromatase assay and the H295R steroidogenesis assay. Based on what is known about the structure of compounds that bind the ER and AR, it was predicted with a high level of certainty that glyphosate would not be a ligand for the ER and AR nor alter steroidogenesis (Schmieder et al. 2003a, b; Schmieder et al. 2004, Blair et al., 2000; Nishihara et al., 2000; Kojima et al, 2004; Fang et at al., 2003; Devillers et al., 2009; Hecker et al, 2011).

Glyphosate has low oral absorption and is rapidly eliminated essentially unmetabolized (Williams et al 2000). Therefore, the potential for systemic exposures to endocrine tissues is extremely low for glyphosate. Results from the Tier 1 Hershberger and Uterotrophic assays with glyphosate demonstrated no impact on estrogenic, androgenic, or anti-androgenic endpoints at the limit dose of 1000 mg/kg/day. Consistent with the results of the multigenerational studies

(BVL 2013; William et al, 2000), there was no evidence of any estrogenic, anti-estrogenic androgenic, anti-androgenic effects on pubertal development or thyroid function up to the limit dose of 1000 mg/kg/day. In accord with the results of the Tier 1 *in vitro* assays, there were also no definitive findings in the glyphosate subchronic, chronic, developmental and reproductive toxicity studies conducted for global registrations that would indicate an endocrine-modulating effect (Williams et al. 2000, Williams et al. 2012; Giesy et al. 2000; WHO/FAO 2004). These repeat dose *in vivo* toxicology studies had extended exposure periods encompassing various stages of endocrine development and did not detect endocrinopathies with histopathological assessment and endocrine organ weight data (Carney *et al.,* 1997; Stevens *et al.,* 1997, 1998; Harvey and Johnson, 2002).

Over the past four decades, in-depth reviews on the safety of glyphosate have been conducted by regulatory agencies and scientific institutions worldwide and concluded that there is no indication glyphosate has endocrine activity. The U.S. EPA (1998) reviewed the subchronic and chronic mammalian studies for glyphosate and concluded that there was no evidence to suggest that glyphosate produces endocrine-modulating effects. In a comprehensive review of the standard mammalian toxicology studies, Williams et al., (2000) also concluded that glyphosate does not have the potential to produce adverse effects on endocrine systems in humans or other mammals and the Institute of Environment and Health (IEH, 2005) lists glyphosate as a substance with no evidence of potential endocrine-disrupting effects. In a recent review of the standard mammalian and wildlife toxicology studies by ECETOC (2009), it was also concluded that glyphosate is not an endocrine disruptor.

In addition to the *in vivo* mammalian assays, the Tier 1 EDSP battery includes two assays with wildlife species. Results from the amphibian metamorphosis assay demonstrated that

glyphosate did not impact thyroid structure or interfere with the function of the amphibian hypothalamic-pituitary-thyroid (HPT) axis up to the highest concentration tested of 90 mg/L. This result is consistent with the findings from the two pubertal assays and from a multigenerational study that evaluated thyroid structure and function (U.S. EPA, 1993). Results from the fish short-term reproduction assay showed no evidence of estrogenic, androgenic or hypothalamic-pituitary-gonadal (HPG) axis effects up to the highest concentration tested of 30 mg/L. This result is consistent with results from the other Tier 1 assays and from a fish full life-cycle study which has a NOEC at the highest tested concentration of 26 mg/L based upon no adverse impacts on survival, growth and reproduction (U.S. EPA, 1993).

Recently, EPA completed their review of the Tier 1 EDSP screening battery for glyphosate (U.S. EPA, 2015). EPA concluded for glyphosate, based on weight of evidence considerations using OSRI that included guideline-compliant studies, that there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways and that Tier 2 EDSP testing is not recommended.

Nonmonotonic dose response curves (NMDRCs) are common after Estrogen or Androgen signaling pathway disruption. Fact or Falderal? by: Leon Earl Gray Jr

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 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 screening and multigenerational toxicity studies.

This presentation discussed LNT, threshold and NMDRCs responses from case studies of chemicals that disrupt reproductive development and function via the androgen (A) and estrogen (E) signaling pathways and includes *in vitro* and *in vivo* multigenerational data. The literature was selected to address several specific questions including:

- What is the shape of the dose response curve over a broad range of doses?
- What is the sensitivity of *in vivo* endpoints to low doses of chemicals that disrupt A and E signaling pathways?
- If NMDRC responses were detected, were these adverse effects and did they occur in the low dose region of the dose response curve?
- What is the potential impact of LNT or NMDRC responses on chemical screening and testing for E and A disruption?

The objective of the literature review was to critically evaluate the reproductive and developmental toxicity data from well executed studies in this field to address concerns that current screening and multigenerational reproductive test guidelines are missing adverse low dose effects of EDs because they routinely induce nonmonotonic adverse effects at low dose. The literature was searched on a chemical-by-chemical basis and included chemicals that disrupted key events in the E and A signaling pathways.

Endocrine disrupting chemicals acting via the following adverse outcome pathways were reviewed to determine the shape of the dose response in the "Low" Dose Range.

Androgen signaling pathway:

- AR antagonists
- Steroid hormone synthesis inhibitors
- Pesticides that disrupt the androgen signalling pathway via multiple mechanisms of toxicity
- Androgen agonists
- Selective androgen receptor agonists (SARMs)
- AhR agonist – 2,3,7,8 TCDD

Estrogen signaling pathway:

- Estrogens
- Selective estrogen receptor agonists (SERMs)
- Aromatase inhibitors

Some studies considered for review were found using Pub Med, or Google search engines while others were selected from extensive literature reviews published in peer-reviewed publications and regulatory agencies guidance or risk assessment documents.

The characteristics for studies included in the review for threshold, linear no threshold, or non-monotonic dose responses were:

- Measured multiple endpoints related to disruption of the estrogen or androgen signaling pathways
- Preferred-Reproductive, one or multigenerational studies
- Preferred – oral administration – diet or gavage
- Included some oral and injection studies of ER or AR mediated gene expression

- Included a broad range of dosage levels from "low" to "high"
    - Definitions of "Low Dose" used in the review
        - ng/kg for chemicals like EE2 and E2, µg/kg for pesticides and toxic substances, or
        - A dose below the reported NOEL
- Preferred – 6 or more doseage levels, but no less than 4 dose levels (three treated groups and a control group)
- Primarily rodent studies also includes some porcine and human studies
- Published literature and Regulatory Agency and NTP documents (and large supplemental files)
- Thousands of papers considered, selected more than 200 *in vivo* studies
    - >70 of which had 6 or more dose levles
    - >40 for the Androgen signaling pathway
    - >30 for the Estrogen signaling pathway

My current conclusions based upon the review of this literature are: 1) EDCs appear to induce some LNT effects *in vivo*. 2) NMDRCs are biologically plausible and occur frequently *in vitro*, but these often occur at high concentrations of estrogens or androgens that are not relevant *in vivo*. 3) It appears that NMRDCs are more common in short- versus long-term exposures, with upstream, mechanistic events versus downstream phenotypic effects. 4) The shape of the dose response curve for an EDC can be affected by several factors, including (but not limited to life stage, route of exposure, target tissue, species differences in E and A pathways or ADME, gut microbiome, and/or concurrent exposure to other chemicals or nonchemical stressors. 5) A few adverse effects of EDs are non-monotonic, but often other effects displaying monotonic

responses occur at lower dosage levels. 6) A number of robust multigenerational studies of estrogens and antiandrogens have been executed and NMDRCs were uncommon at low dosage levels. 7) Multigenerational test guidelines can be enhanced on a case-by-case basis to improve the sensitivity to low dose effects of some EDCs. 8) Additional data need to be examined from robust, multigenerational studies using a broad range of dosage levels for other pathways.

Modernizing Problem Formulation for Risk Assessment: Potency and Mass Action Govern Endocrine Activity; Christopher J. Borgert

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. As a result, HI processes do not address the types of mechanistic data that arise in identifying potential endocrine activity, and unlike basic sciences, have not been modernized to keep pace with advancements in biological and pharmacological understanding. The thesis proffered here is that if risk assessments for endocrine active substances are to claim a basis in modern science, the problem formulation phase must be modernized so that HI is based on potency thresholds rather than a presumption of effects based on the mere identification of potential endocrine activity.

The need for recognizing potency thresholds in the identification of endocrine hazards is firmly grounded in fundamental principles of endocrine pharmacology, which have been established over decades of experimental and clinical research. 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 together determine receptor occupancy and activation state in target cells. Discrimination based on potency can be theoretically-derived and corroborated by experimentally and clinically observable potency thresholds, without which normal physiological functions would be impossible (Borgert et al. 2013; 2012). Although it has been argued that because the endocrine system is basally activated by endogenous hormones, very small amounts of low-potency chemicals could alter its function, simple receptor occupancy calculations reveal that in contrast, trillions of molecules would be required to change receptor occupancy by any measurable degree (Borgert et al., 2013). The requirement for a sufficient change in receptor occupancy and cellular activation state, both of which depend on potency and mass action, forms the theoretical basis for potency thresholds derived directly from established principles of endocrine pharmacology.

Potency thresholds for the induction of endocrine-mediated effects can be estimated empirically from an understanding of the differential potency of endogenous hormones (or their pharmaceutical agonists and antagonists) versus endogenous products of metabolism or essential nutrients that may interact with the hormone's receptor but which lack hormonal function (Borgert et al. 2013). An example of such differential potency is seen with pharmaceutical estrogens, which exhibit potencies within one to two orders of magnitude of the primary endogenous estrogen, 17-β-estradiol, versus both aromatizable and non-aromatizable androgens, which exhibit potencies five to six orders of magnitude less than that of the endogenous estrogen (ICCVAM, 2011; Chen et al. 2005; Borgert et al. 2013). While the effects of many androgens

662 on estrogen-sensitive tissues could occur via conversion to estradiol by aromatase, this
663 conversion does not occur to any appreciable extent for non-aromatizable androgens. Although
664 androgens are also uterotrophic, albeit at high doses, the effect is blocked by cyproterone but not
665 by ICI-182,780, and thus appears to be an anabolic effect mediated by uterine androgen rather
666 than by estrogen receptors (Beri et al., 1998; Schmidt et al. 1979; 1976). A second example
667 includes essential fatty acids, which exhibit low-potency estrogenic and anti-estrogenic activity
668 *in vitro*, but which fail to elicit clinically identifiable estrogenic activity even at high doses
669 (reviewed in Borgert et al. 2013). Several phytoestrogens exhibit potencies intermediate
670 between the endogenous or pharmaceutical estrogens and androgens (ICCVAM, 2011; Ranhotra
671 & Teng, 2005; Kim et al., 2005). The high-dose estrogenic activity of phytoestrogens in sheep
672 (Adams, 1995) versus their lack of apparent clinical effect in women (Cline et al., 2001) suggests
673 that these natural compounds could be used to define a potency threshold for estrogenic hazard,
674 similar to their use as a benchmark for activity-exposure profiling in prioritizing chemicals for
675 endocrine screening (Becker et al., 2015). Based on this example, the potency threshold for
676 defining an estrogenic hazard could be set conservatively at four orders of magnitude below the
677 potency of the endogenous hormone 17-β-estradiol.

678 Requirements for using the maximum tolerated dose concept based on body weight
679 reductions and other measures of overt toxicity have been a primary deterrent to modernizing the
680 HI step of risk assessment for cancer and general toxicity endpoints, but can be remedied by use
681 of toxicokinetics in dose setting (Saghir et al., 2012) and articulating hypothesized modes of
682 action in problem formulation (Borgert et al. 2015). For potentially endocrine-active substances,
683 arguments favoring a no-threshold assumption based on fluctuating and heightened hormonal
684 sensitivity during some life stages should be addressed in order to justify modernizing HI to

comport with well established principles of endocrine pharmacology that rely on thresholds of potency. While it is clear that sensitivities to hormones vary during different life stages, it is also clear that the mechanisms enabling discrimination of molecular potency fluctuate accordingly, thus preserving the ability of the endocrine system to distinguish the biological signals produced by potent ligands such as hormones and pharmaceuticals from spurious molecular interactions with low-potency substances such as normal products of metabolism and nutrients (reviewed in Borgert et al., 2013). Hence, while it is important to consider exposures to sensitive life stages when assessing risks, identifying endocrine hazards depends on the differential potencies of hormones versus molecules that interact with insufficient potency to convey or interrupt endocrine signals regardless of life stage sensitivity.

In summary, 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. These properties are well established and used clinically in endocrine pharmacology, but have not yet been incorporated into HI for risk assessment. Unless the HI step is modernized to incorporate these well-established principles and phenomena, false hazards will be proposed, followed by the needless expenditure of animals, effort and resources to calculate and manage theoretical risks that could never manifest as adversity. Without the modernization step proposed here, hazard identification based on endocrine screening methods would conceivably identify substances as potential estrogens that, in fact, present as little estrogenic hazard (*i.e.,* none) as non-aromatizable androgens and essential fatty acids. The derivation of hormone-specific potency thresholds for defining potential endocrine hazards is a theoretically sound and empirically supportable method for averting such problems.

CONCLUSION

Discussion at the Focused Topic Meeting made it clear that an overwhelming majority of attendees believed that risk assessment and management of EDCs can be conducted in a safe and scientifically sound manner, although it was pointed out that one non-EU jurisdiction (Brazil) is also proposing to regulate EDCs by their hazard alone. The rationale for this policy was primarily based on political necessity due to resource limitations. There was strong support for the proposed SETAC Pellston Workshop ™ , (proposed by Matthiessen in this publication), as a rational way forward to further enhance discussion on EDCs and potentially develop guidance for environmental hazard and risk assessment approaches of endocrine active substance. This workshop was held in early February 2016 and publications that emanated from this workshop are currently in review for publication by IEAM[3] . Furthermore, the following outreach statement on EDCs was drafted as an outcome to Session four of the SETAC North America Focused Topic Meeting: Endocrine Disruption.

SETAC FOCUSED TOPIC MEETING ON ENDOCRINE DISRUPTING CHEMICALS: OUTREACH STATEMENT

More than 200 participants representing industry, government, and academia from ten countries attended a SETAC North America Focused Topic Meeting (FTM) on February 4-6, 2014 dealing with the issue of *"Endocrine Disruption: Chemical Testing, Risk Assessment Approaches and Implications."* The primary focus of the FTM was to address the dichotomy of approaches evolving for the management of endocrine disrupting chemicals (EDCs). EDCs are defined as exogenous chemicals or mixtures that can alter the function(s) of the endocrine system

and consequently cause adverse health effects in an intact organism, its progeny, or (sub) populations (see also SETAC Tip: http://www.setac.org/resource/resmgr/Publications_and_Resources/Endo-TIP.pdf).

It is possible that as many as 50,000 chemicals could require assessment for their endocrine disruption potential. Results from those assessments will influence decisions concerning new chemical approvals and the handling of existing chemicals in commerce. In the US, Canada and Japan, the approach is risk-based, incorporating both the inherent hazards and exposure potential when determining risks posed by suspected EDCs. In contrast, in Europe, a hazard-based approach is being discussed because there is concern among some toxicologists and endocrinologists that traditional risk assessment may not always be appropriate when considering unresolved issues including low-dose or non-threshold effects and portions of the life cycle sensitive to exposure. In the hazard-based approach, the primary focus is the intrinsic endocrine hazard of a chemical and not the effect concentration or environmental concentrations of the chemical in question.

Some attendees supported the hazard-based approach because it is precautionary in nature. They were not convinced that traditional risk assessment covers the uncertainties connected to potential no-threshold, low dose, or sensitive periods of exposure and response to endocrine disruptors. However, the majority of attendees at the FTM supported the concept that EDC assessments should consider environmentally–relevant exposures. It was also recognized that interactions of chemicals with endocrine receptors or alterations in endocrine response do not always result in irreversible adverse outcomes, and that linkages between endocrine mediated responses and adverse outcomes such as malformations, growth, reproduction and development

753 must be established. This was considered important despite the fact that these assessments are
754 more costly and time consuming to conduct.

755 The FTM presented an opportunity to publically recognize some of the controversies
756 surrounding the developing science around EDCs and to further the debate concerning hazard-
757 and risk-based approaches. At this time there is no agreement on the manner by which EDCs
758 should be regulated although most participants were convinced that efforts to advance our
759 understanding of the potential impacts of EDCs need to be based on a systematic review of all
760 available information and that agreed upon criteria be developed to evaluate these data. In the
761 end, the FTM recommended the need for meaningful dialog between the proponents of risk and
762 hazard based approaches to evaluate EDCs as this will be critical in assisting both the public and
763 regulators on an issue that may impact both humans and wildlife.

764 As a follow up to the discussions held at the FTM and a preceding meeting in Brussels in
765 2012, a SETAC Pellston workshop was proposed to develop scientific case studies of both
766 environmental hazard and risk assessment approaches applied to EDCs. The idea was to use real-
767 world data to evaluate different assessment method which, conducted rigorously by global
768 experts on EDS, would give rised to authorative guidance to regulators. This workshop has been
769 held in the meantime.[3]

[3]Note from the Guest Editor: The SETAC Pellston Workshop™ 'Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Substances (EHRA)' was held from 31st January to 5th February 2016 in Pensacola, Florida, USA. The primary aim of the workshop was to provide objective advice, based on current scientific understanding, to regulators and policy makers, whether in industry, government or academia; the aim being to make considered, informed decisions on whether to select an ecotoxicological hazard- or a risk-based approach for regulating a given endocrine-disrupting substance (EDS) under review. The workshop additionally considered recent developments in the identification of EDS. Case studies were undertaken on six endocrine active substances (EAS – not necessarily proven EDS), that are representative of a range of perturbations of endocrine system and considered to be data-rich in relevant information at multiple biological levels of organisation for one or more ecologically-relevant taxa. The workshop was successful in developing consensus. Scientific papers are currently in review for publication by IEAM.

DISCLAIMERS

The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

The manuscript has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the USEPA and mention of trade names or commercial products does not constitute endorsement or recommendation for use by USEPA.

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